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by

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Summary

It is recognized that species of fungi vary in their ability to utilize different sugars for growth and other purposes. However, it is difficult to compare the results in the literature because different experimental methods and different isolates were used. The experiments reported in this bulletin were made under uniform environmental conditions, and growth was measured in all instances by weighing the mycelium produced.

The amount of mycelium produced by 57 species of fungi when grown on 12 sugars was determined for various periods of incubation. Much variation in the *rate* of utilization of the various sugars was found. Some sugars appeared to be utilized at once, others were utilized only after the fungus had been in contact with a particular sugar for some time. The time of incubation is one of the more important experimental variables.

The 12 sugars used in the first experiments reported were: D-glucose, D-fructose, D-mannose, D-galactose, L-sorbose (hexoses), L-arabinose, D-xylose (pentoses), maltose, sucrose, lactose, cellobiose (disaccharides) and raffinose (trisaccharide). All of the fungi tested utilized glucose, and with one or two exceptions the closely related sugars, fructose and mannose. Galactose was utilized slowly by about half of the fungi and poorly by two species. Sorbose, an uncommon sugar in nature, is slowly or poorly utilized by many fungi. Of the two pentoses tested, xylose was the better sugar for most fungi. A few species utilized arabinose better than xylose. All of the tested species except one utilized cellobiose, whereas five species grew slowly or poorly on maltose. At least six utilized sucrose poorly if at all. Lactose was unsatisfactory for more species than any of the other disaccharides tested. About one-third of the species tested made slow or poor growth on raffinose.

Failure of certain species to utilize various oligosaccharides may be correlated with failure of the fungus to hydrolize the sugar in question. Those fungi that failed to utilize maltose, sucrose, lactose, cellobiose, and raffinose grew upon the simple sugar or mixtures of simple sugars which

result from the complete hydrolysis of these oligosaccharides. These observations are not proof that oligosaccharides are hydrolyzed before utilization by fungi which utilize complex sugars readily.

Fungi rarely, if ever, come in contact with a single carbohydrate in nature. Some evidence was obtained that the disaccharides sucrose and melibiose, which are not utilized by some fungi when they are the only sugars in the medium, are utilized in the presence of a utilizable simple sugar.

Extensive studies were made on the utilization of sorbose, alone and in mixtures with other sugars. When sorbose is the only sugar in the medium it is utilized readily by some fungi and poorly or not at all by others. When mixed with glucose, sucrose, or maltose, sorbose inhibits the utilization of these sugars by some fungi which do not utilize sorbose. The inhibition is greater in maltose than in glucose media and often is overcome by prolonged incubation. It is much more pronounced in most fungi during early stages of growth.

The inhibition was shown not to be due to the increased amount of sugar in media containing mixed sugars. The relative amounts of sorbose and maltose or glucose was an important factor determining inhibition.

Sorbitol, the related sugar alcohol, was a poor carbon source for most fungi tested but was not inhibitory. Experiments showed that the sorbose inhibition was not due to a toxic product formed during autoclaving. It was not overcome by the addition of a mixture of vitamins to the medium. The pH of the medium had only a slight modifying effect.

The addition of malt extract partially overcame the inhibition caused by sorbose. The source of nitrogen in the medium had a marked effect on the degree of inhibition but not all of the species tested responded to the same nitrogen source. The inhibition was more severe at temperatures of 30° C. or above than at lower temperatures, and sometimes was not evident at 20° C.

The general effect of sorbose on fungi is to restrict the extension of mycelium. This often results in greatly increased branching, which may or may not be reflected in dry weight produced. Fungi highly sensitive to sorbose may have the majority of the hyphal tips killed in a sorbose medium.

It is generally concluded that growth of a fungus on a mixture of sugars cannot be predicted on the basis of its utilization of the separate sugars. The utilization of sugars (either alone or in mixtures) is modified by a number of factors, which include other constituents of the medium, the environment, and time.

Introduction

The fungi play an important role in the economy of nature by destroying the organic materials synthesized by green plants and other organisms. The biologically essential elements are thus returned to the storehouse of nature for reuse by other organisms. In a general way, the processes of synthesis and destruction in the biosphere are in dynamic equilibrium. The carbohydrates are synthesized by green plants in large amounts. These compounds range from low molecular weight water soluble sugars to highly polymerized insoluble compounds such as starch and cellulose. In nature, the fungi rarely, if ever, come in contact with a single sugar. The behavior of fungi in the presence of mixed sugars is not always predictable from their behavior on the single sugars comprising the mixture.

Detailed information is available on the utilization of sugars and related compounds by a few fungi. The most extensive studies appear to be those of Steinberg (1939, 1942) on *Aspergillus niger,* Tamiya (1932) on *A. oryzae,* and Margolin, (1942), who studied the growth of twenty-one fungi on the common mono- and disaccharides. A considerable number of the Saprolegniaceae have been studied from the standpoint of sugar utilization. Volkonsky (1933, 1934) found that the Saprolegniaceae, with the exception of a species of *Aphanomyces,* utilized only glucose or carbohydrates containing glucose alone (maltose and dextrin) when glycine was used as the source of nitrogen; the closely related sugar fructose was not utilized. When peptone was used as the source of nitrogen other sugars were utilized. Bhargava (1945) studied the sugars utilized by five species of Saprolegniaceae. Cantino (1950) has reviewed the nutrition of the aquatic fungi, including the available information on sugar utilization.

Van Neil (1944) has said that if a microorganism is able to utilize any sugar, glucose is the sugar used. Yet, some fungi have been reported as being unable to utilize carbohydrates (Schade, 1940: Schade and Thimann, 1940). Such instances appear to be rare.

An examination of the scattered and fragmentary information in the literature would greatly add to the list of species able to utilize a few of the common sugars such as glucose, maltose, and sucrose. Much of this information is incidental to other investigations, and the experimental methods used by the various investigators differ widely. A portion of the available information on sugar utilization has been summarized and is to be published in the *Handbook of Biological Data.* Only 84 species are included in this summary, many of which were studied in this laboratory.

The negative results found in the literature before 1935-40 are to be viewed with caution because it was not generally recognized that many fungi require an exogenous source of vitamins for growth. The common failure to allow a sufficiently long period of incubation greatly reduces the value of many papers, for the reader is left uncertain whether slow utilization or total inability to utilize a specific sugar is involved.

The general purpose of this work was to extend our knowledge and increase our understanding of the utilization of sugars by fungi. Two lines of investigation were followed. The first was to determine the ability of 57 fungi to utilize 12 sugars for growth, and to determine the relative rates of growth of the test fungi on these sugars when used singly. Some supplementary experiments were made with fungi that failed to utilize certain oligosaccharides. The second part of this bulletin reports the results of experiments on the effect of L-sorbose on the utilization of certain other sugars.

Part I
RATE AND AMOUNT OF GROWTH OF 57 FUNGI UPON 12 SUGARS

Materials and Methods

The cultures used in this work were taken from the stock culture collection of fungi maintained by the Department of Plant Pathology, Bacteriology, and Entomology, West Virginia University. The origin of these cultures is available to any interested investigator.

In the experiments reported in Part I of this bulletin the sugars were used at a rate that supplied 10 grams of carbon per liter of medium[1]. Any exceptions are noted in connection with individual experiments (Tables 2, 3 and 4). The sugar concentrations used in the experiments reported in Part II of this bulletin are noted in connection with the individual experiments. The basal medium used in this work had the following composition.

Basal Medium

Asparagine monohydrate	2.0 g.
KH_2PO_4	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
Micro elements[2]	2 ml.
Thiamine hydrochloride	100 μg.
Biotin	5 μg.
Double distilled water, to make	1000 ml.
pH before autoclaving	6.0

The salts used in preparing the basal medium were of analytical reagent grade to meet A.C.S. standards. The asparagine used was a Difco product. A commercial grade of anhydrous glucose was used. The sucrose, maltose, and D-xylose were purchased from Difco. The other sugars used were Eastman white label products. No attempt was made to further purify the sugars.

[1]To furnish 10 g. of carbon the following amounts of the various sugars are required: D-glucose, D-fructose, D-mannose, D-galactose, L-sorbose, L-arabinose, D-xylose, maltose monohydrate and lactose monohydrate 25 g.; sucrose and cellobiose 23.7 g.; and raffinose pentahydrate, 27.3 g.

[2]The micro element solution was prepared by dissolving $Fe(NO_3)_3 \cdot 9H_2O$, 723.5 mg; $ZnSO_4 \cdot 7H_2O$, 439.8 mg.; and $MnSO_4 \cdot 4H_2O$, 203.0 mg. in distilled water acidified with sufficient sulfuric acid to yield a clear solution and made up to a volume of 1 liter. Each ml. of the above micro element solution contains 0.1 mg. each of iron and zinc and 0.05 mg. of manganese.

The basal medium used in this work was adequate to support fair to good growth of most of the fungi tested, provided that the sugar supplied was utilized. One fungus, *Coemansia interrupta,* produced only a small amount of mycelium in comparison with the other species. However, this fungus makes only sparse growth on other media we have used. It is realized that the basal medium is not optimal for all species, but an attempt to provide an optimum medium for each fungus would have multiplied the work to a prohibitive degree. The use of other media or other ways of handling the cultures would have no doubt modified the results.

The basal medium usually was prepared in lots of 6 to 12 liters. The pH was adjusted to 6.0 and aliquots added to weighed amounts of the 12 sugars. This procedure insured that the basal medium for any one experiment with a given fungus was the same. Pyrex Erlenmeyer flasks of 250 ml. capacity were used as culture vessels, each flask containing 25 ml. of medium. Media were sterilized by autoclaving for 15 minutes at 15 pounds steam pressure. The flasks were removed from the autoclave 5 minutes after the steam pressure was down. This was done to avoid excessive caramelization of the sugars. The ketose sugars, L-sorbose and D-fructose darkened more than other sugars on autoclaving. It is known from the work of Margolin (1942) and others that the method of sterilizing media may affect the rate and amount of growth of some fungi. In view of the almost universal use of autoclaving to sterilize media and the simplicity of the method, it was chosen for this work. Caution should be used in extrapolating the results obtained when sugars are autoclaved to those where milder methods of sterilization are used. This caution is pertinent to any study of the nutritional aspects of parasitism. In their natural habitat fungi grow on substrata that have not been subjected to heat.

The inoculum was grown in Petri dishes on malt extract agar (20 grams each of malt extract and agar). Small pieces of the mycelium and agar, approximately 2 by 2 mm. were used to inoculate the flasks.

The fungi were grown in constant temperature rooms at 25± 1° C. These rooms were illuminated 12 hours each day by fluorescent lamps.

Time of incubation is a most important condition in studies of this kind. Since fungi utilize sugars at different rates it was considered essential that results be obtained for different periods of incubation. The general plan of the experiments was to use four flasks of each sugar medium per fungus in a preliminary trial. Following this a more extensive experiment was made using 6 to 8 flasks of each medium. If a sugar was utilized poorly or slowly, additional flasks were included, or supplementary experiments with certain sugars were made. The

final time of incubation was such that the fungus had attained maximum weight on one or more of the 12 sugars. In many instances much longer periods were used. After cultures have attained maximum weight they lose weight due to autolysis. This is a certain indication that the period of maximum growth is past. In many instances where sugars were utilized poorly or slowly it is probable that maximum weight was not attained.

The mycelium was harvested by filtering on a fine mesh cloth. The mycelium was transferred to aluminum cups of known weight and dried overnight at 100° C. Weighing was done on an analytical balance, the results reported as milligrams of dry mycelium. In general, the weights of duplicate cultures agreed satisfactorily. The data reported in the tables are the averages of two duplicate cultures.

All of the experiments were repeated, but the times of incubation were not always the same in both experiments. The amount of early growth varied somewhat in the two experiments, yet the general conclusions regarding the rate and amount of growth were the same in both experiments. For any one time of harvest, the data for the 12 sugars are taken from one experiment.

Experimental Results

In the experimental work reported in Part I of this bulletin the following subjects were investigated: (1) The rate and amount of growth of 57 fungi when 12 sugars were added singly to the basal medium in such amounts that each liter of medium contained 10 grams of carbon from the sugars; each 25 ml. aliquot contained 0.25 grams of sugar-carbon. (2) The rate and amount of growth of a number of fungi that grew slowly or poorly on sucrose, lactose, and raffinose also were determined on mixtures of the sugars which result from the hydrolysis of these oligosaccharides.

The detailed results presented in Table 1 permit an evaluation of the rate and amount of growth by 57 fungi on 12 sugars. Failure to utilize or slow utilization of the monosaccharides used must be referred to the structure and configuration of these sugars. An additional factor may operate in failure to utilize an oligosaccharide. If the first step in the utilization of an oligosaccharide is hydrolysis, and the hydrolytic products are utilized, the failure of some of the test fungi to utilize certain oligosaccharides may be ascribed to failure in performing this hydrolysis. Since maltose and cellobiose yield only glucose on hydrolysis the necessary internal control is found in Table 1.

TABLE 1. MILLIGRAMS OF DRY MYCELIUM PRODUCED BY 57 FUNGI WHEN GROWN ON 12 SUGARS AS A FUNCTION OF THE TIME OF INCUBATION. DATA ARE AVERAGE VALUES FOR DUPLICATE CULTURES

Species	Days of Incubation	D-glucose	D-fructose	D-mannose	D-galactose	L-sorbose	L-arabinose	D-xylose	Maltose	Sucrose	Lactose	Cellobiose	Raffinose
Alternaria solani	7	82	77	83	76	13	105	74	123	43	40	45	31
	15	196	120	153	169	55	206	190	180	117	86	125	137
	23	205	193	200	207	107	190	160	175	186	73	210	213
	35										118		
Alternaria tomato	7	25	43	15	16	3	15	25	50	38	30	24	14
	11	86	113	34	30	5	15	42	40	42	20	46	41
	14	129	123	111	39	4	33	60	98	61	34	36	60
Aspergillus clavatus	9	223	190	212	58	16	42	169	203	187	50	200	88
	14	212	193		103	32	154	206	185	189	213	204	88
	27			183		51			170		186		91
	40					182							
Aspergillus elegans	5	206	189	199	148	37	155	180	211	243	22	115	209
	14	200	179	188	164	37	160	184	188	226	54	205	207
	39					93					146		
Aspergillus niger	4	240	200	213	78	29	36	165	166	206	10	60	167
	7	252	205	279	136	59	166	217	264	256	23	207	171
	19	210	186	196	189	182	211	212	202	209	55	108	204
Aspergillus rugulosus	3	40	20	26	19	5	12	12	37	33	9	27	26
	6	154	79	120	92	20	75	86	132	163	41	60	138
	13	375	376	375	327	136	236	354	336	420	164	269	164
	26					295					278		250
Botrytis cinerea	5	133	115	147	79	9	19	52	121	103	26	67	53
	9	376	252		226	138	85	172	229	294	270	244	183
	21	242	209		189	178	176	185	222	219	240	199	198
Chaetomium convolutum	11	55	60	60	48	1	68	66	77	61	18	54	20
	41	152	190	143	152	8	119	160	155	142	151	136	104

Chaetomium globosum	4	51	55	42	37	4	6	39	43	20	7	32	21
	7	254	252	361	123	6	16	78	173	166	20	83	120
	12	328	310	292	255	13	59	105	253	270	38	161	94
	21				283	35	123	170			84		139
	39				179	58	219	165			147		137
Choanephora curcurbitarum	5	73	74	38	77	4	37	9	56	12	6	45	8
	8	83	96	91	126	3	30	19	105	20	12	79	16
	16	72	71	68	89	4	73	64	78	21	19	83	18
Colletotrichum lindemuthianum	11	49	88	87	40	2	34	33	38	37	44	33	34
	16	113	132	132	113	4	51	30	71	108	100	112	110
	30	141	129	116	148	12	128	175	137	141	161	118	150
	54					20							
Colletotrichum phomoides	5	155	67	115	107	2	32	33	106	170	40	53	110
	12	225	286	242	270	11	195	210	263	196	189	239	238
	22	157	173	184	205	30	179	187	201	159	223	219	188
Collybia velutipes	9	65	78	55	36	4	39	20	34	64	21	33	29
	21	148	135	159	47	18	161	172	198	183	69	122	115
	59				213	78					195		
Coemansia interrupta	14	5	5	5	6	3	5	5	8	10	5	8	5
	31	10	5	9	8	6	5	5	7	8	10	8	5
	40	12	11	9	11	3	13	13	4	10	15	5	4
Cordyceps militaris	5	52	18	36	38	3	4	1	31	67	11	19	15
	7	233	63	135	84	2	9	4	118	171	23	79	39
	13	204	234	251	148				228	247		210	
	20					11	81	25			20		53
Cordana sp.	11	45	38	51	40	6	30	57	16	17	20	53	13
	19	168	174	162	177	8	96	181	21	11	54	144	12
	25	149	158	153	170	6	163	172	21	16	81	150	15
	42					19			114	19	225		18
	49					31			185	61	213		29
Cytospora intermedia	13	38	14	34	30	6	19	23	63	25	9	46	17
	25	65	57	63	52	18	40	53	93	52	16	69	43
	40	68	89	77	108	15	65	123	108	103	63	76	85
Dendrophoma obscurans*	8	39	24	7	44	4	17	39	41	27	21	54	11
	15	93	80	60	77	23	58	135	91	88	68	127	41
	26	174	121	104	92	53	83	173	171	170	98	182	68

TABLE 1 (CONTINUED)

Species	Days of Incubation	D-glucose	D-fructose	D-mannose	D-galactose	L-sorbose	L-arabinose	D-xylose	Maltose	Sucrose	Lactose	Cellobiose	Raffinose
Endoconidiophora adiposa	2	71	91	63	71	12	25	9	95	86	6	20	32
	3	154	180	143	150	98	49	6	133	159	15	53	97
	5	165	202	179	228	245	151	29	178	149	13	123	153
	7					186	201	84			26		
Endoconidiophora fagaccarum	7	45	23	82	30	0	5	8	25	63	4	37	23
	14	164	53	171	78	1	31	95	156	141	10	147	56
	22		157	83	126	6	85	181			30		88
	35		137			16	159	129			47		80
Endoconidiophora fimbriata	5	118	50	82	35	0	1	5	66	85	3	21	14
	11	110	114	122	143	2	19	8	132	147	6	97	14
	18		120			2	71	118			5		11
	31					66					8		5
Endoconidiophora virescens	3	90	20	64	27	2	9	3	65	76	4	26	70
	7	215	254	234	176	2	35	1	246	256	26	229	141
	11	204	240	210	180	34	95	19	227	238	28	196	127
Endothia parasitica	7	205	169	109	39	12	14	58	166	169	8	187	146
	14	227	249	161	70	56	31	209	201	257	10	242	208
	21		223	129	177	127					9		215
	39				138	109					11		
Fusarium conglutinans	3	90	39	90	73	12	6	19	85	47	22	53	43
	6	277	231	238	197	265	191	231	264	237	51	124	136
	10	265	240	246	244	307	255	247	348	253	113	247	151
Fusarium culmorum	4	78	60	57	74	18	28	68	112	56	22	17	36
	12	146	171	145	144	167	154	139	166	141	30	53	117
	17					156	157	146		119	58		104
	40										75		
Fusarium lycopersici	4	153	124	153	106	37	126	184	159	84	30	71	42
	7	266	271	267	224	265	250	258	244	244	112	144	164
	14										172	225	

Fusarium	4	185	194	206	179	145	156	188	186	191	33	99	187
medicaginis	11	167	213	204	193	199	176	187	198	147	121	168	200
	30					125					107		
Fusarium	4	88	88	100	102	5	73	108	98	79	37	21	59
nivale	11	140	155	155	186	118	170	145	163	141	124	146	160
	16										117	141	
Fusarium	4	17	31	80	25	4	14	8	140	100	14	39	38
niveum	7	172	171	199	160	23	151	160	242	198	23	221	191
	15	181	195	204	215	216	213	181	190	191	35	223	197
Fusarium	4	155	169	147	153	175	87	150	163	109	27	27	117
tracheiphilum	10	215	234	231	216	257	240	236	236	194	96	208	226
	25										196		
Gliomastix	4	44	71	95	64	12	50	94	129	83	65	67	46
convoluta	8	250	271	253	245	160	246	253	252	211	186	186	45
	14	189	209	203	190	227	164	160	183	197	217	233	66
Glomerella	5	270	150	175	118	11	97	162	256	213	29	89	151
cingulata	10	285	249	301	216	123	214	288	254	260	135	250	248
	16	176	243	230	217	195	176	164	165	252	256	238	192
Helminthosporium	5	184	195	168	76	12	26	45	229	180	29	44	113
sativum	14	217	153	236	250	62	151	306	195	205	270	209	244
	227					170	255						
Lenzites	21	24	30	68	37	80	11	125	16	38	4	7	5
sacpiaria	34	180	111	265	112	204	18	256	309	80	71	110	9
	59	236	85		188	243	37	240	155	245	269	230	92
Lenzites	11	78	16	125	27	6	7	22	43	23	33	30	8
trabea	18	140	44	118	53	22	18	77	148	108	133	152	16
	25		139		61	57	44						58
	35												129
Melanconium	7	108	120	111	82	11	43	45	94	86	8	126	56
fuligineum	14	181	203	217	158	57	160	191	180	166	15	200	112
	22				170	142		163		185	23		
	35					149			155				151
Monascus	6	128	44	37	24	9	43	30	10	18	7	16	8
purpurea	9	297	96	113	29	9	198	65	16	15	9	73	9
	15	238	225	247	74	14	219	240	43	17	21	238	16
	26	229	224	233		26	220	244	122	17	39	200	29

TABLE 1 (CONTINUED)

Species	Days of Incubation	D-glucose	D-fructose	D-mannose	D-galactose	L-sorbose	L-arabinose	D-xylose	Maltose	Sucrose	Lactose	Cellobiose	Raffinose
Penicillium expansum	3	196	188	175	79	145	71	113	166	212	16	79	161
	5	181	193	202	123	170	65	157	170	176	43	178	155
	10	147	154	172	181	156	115	169	93	146	40	168	160
Penicillium spiculisporum	5	133	96	181	75	7	132	110	28	113	9	47	35
	7	201	213	226	158	13	173	193	53	181	10	188	77
	19	107	118	92	124	159	105	126	192	128	20	128	130
Phoma betae	6	22	56	12	12	1	17	14	86	10	13	17	27
	12	163	123	135	128	24	104	136	136	136	61	89	207
	20		150		167	209	162				144	179	151
	35					150	118				138		
Phycomyces blakesleeanus	5	150	140	166	90	5	64	148	162	71	14	50	7
	8	143	161	173	95	2	146	158	160	145	10	107	7
	15	155	171	165	111	2	150	157		113	4	154	47
	55					4					7		87
*Phytophthora*** *infestans*	30	22	46	8	12	0	8	9	11	8	7	4	8
	42	58	59	11	13	0	7	8	8	8	7	4	7
Polyporus albellus	26	79	40	100	86	11	15	91	52	13	5	47	14
	34	159	134	154	94	17	31	175	110	12	4	99	7
	42					14	43			48		174	16
Polyporus versicolor	7	28	30	26	24	11	10	18	35	32	13	18	23
	16	282	270	282	60	137	33	251	237	254	78	276	182
	32	224	185	202	197	210	108	218	180	200	216	248	162
Rhizoctonia solani	5	276	162	336	227	3	105	122	270	204	69	34	138
	7	464	388	429	386	4	167	312	406	416	272	260	414
	21	311	318	375	320	49	192	383	244	268	277	377	253
Schizophyllum commune	7	41	20	15	28	2	10	18	32	28	20	15	18
	9	339	186	213		6	9	19	55	68	22	18	23
	13	297	224	323	113	12	96	80	141	278	139	110	165
	17	273	235	231	57	15	48	98	283	228	230	288	125
	23					38	131	197					148
	36					52							191

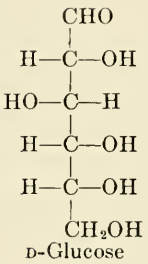
Schizothecium longicolle	5	13	13	15	20	2	3	33	29	17	3	28	7
	9	78	119	88	23	2	2	88	92	19	5	67	6
	18	202	182	218	15	3	3	219	209	22	19	200	10
	30	167	164	198	56	10	13	183	179	24	124	189	20
Sclerotinia sclerotiorum	7	57	39	57	35	2	9	9	7	22	16	11	18
	12	240	149	184	104	4	70	123	100	37	132	114	114
	21	181	245	212	256	4	200	186	189	149	154	183	243
Sordaria fimicola	5	163	77	153	56	5	23	103	155	18	45	153	6
	8	179	119	170	143	5	66	163	216	20	164	207	13
	13	252	267	258	169	2	149	238	286	10	285	285	7
	20					6	222			11			7
Sphaeropsis malorum	5	75	37	67	32	2	6	3	81	90	10	45	21
	12	206	211	204	202	16	188	39	163	173	81	209	200
	19		163		244	39	214	79			52		199
	36					160					190		
Stysanus stemonitis	5	162	37	101	140	2	68	50	96	81	51	62	76
	8	208	154	166	202	2	154	163	189	159	131	160	171
	15	198	168	187	190	3	193	197	224	163	215	201	198
Thielaviopsis basicola	4	123	64	114	73	1	8	1	84	33	3	31	7
	9	178	115	187	229	2	116	1	204	199	6	226	17
	13	159	158	185	216	46	214	2	182	182	9	193	200
Zygorhynchus vuilleminii	2	43	58	37	79	8	26	14	19	38	17	21	19
	6	175	174	192	178	14	39	187	23	192	19	94	26
	12	131	148	162	188	18	39	179	25	148	18	160	100
	34					14	66		115		20		86

*5 mg. inositol was added per liter of medium for this species.
**Cultured at 20° C.

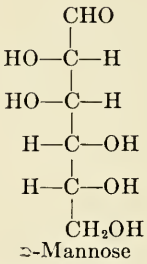
Utilization of Monosaccharides

When a number of fungi are grown on a single sugar only differences between species can be compared. However, when more than one sugar is used, the structure and configuration of the sugars must necessarily be considered. Only such features of sugar chemistry as seem essential to an understanding of the similarities and differences among the sugars used will be presented. Other details concerning the sugars used in this work may be found in texts on carbohydrate chemistry, *e.g.*, Pigman and Goepp (1948).

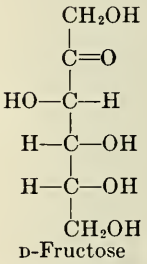
Glucose, Fructose and Mannose: These sugars are closely related chemically, and it will be convenient to consider the utilization of these three sugars together. The simple Fischer projection formulas are shown below*



D-Glucose



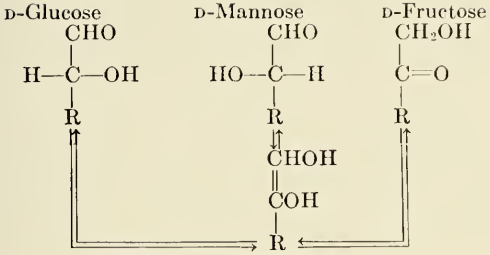
D-Mannose



D-Fructose

The close chemical relationship among these sugars is revealed by the fact that when any one of them is placed in a weak alkaline solution the other two are formed. This isomerization is known as the Lobrey de Bruyn-Alberda van Eckenstein transformation. The formulation of this equilibrium presented below is adapted from that of Sowden and Schaffer (1952). More deep-seated changes also occur when sugars are exposed to alkaline conditions, especially in the presence of oxygen. As the time of exposure to alkaline conditions is extended, more and more of the sugars are converted into compounds which are not fermented by yeast. Fructose is more reactive than either glucose or mannose. Other reducing sugars react similarly to alkali. Englis and Hanahan (1945) have shown that phosphate catalyzes various transformations in glucose on autoclaving. These are excellent reasons for not autoclaving sugars in alkaline media or using alkaline media. The initial pH of media used in this work was sufficiently low to avoid this transformation.

*Many of the formulas listed were taken from *Physiology of the Fungi*, by V. G. Lilly and H. L. Barnett, and reprinted by permission of McGraw-Hill Book Company, Inc., 1951.



Common enol form

A close, but not identical, pattern of utilization was found for glucose, fructose, and mannose. Most of the fungi tested grew approximately at the same rate and produced about the same yield of mycelium on these three sugars. Certain exceptions should be noted.

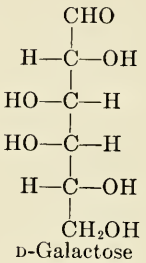
Phytophthora infestans made only a trace of growth on mannose, while the initial rate of growth of *Dendrophoma obscurans* was slow on this sugar. With the exception of *P. infestans* all of the fungi tested utilized glucose, fructose, and mannose. In a number of instances the rate of initial growth on fructose was less than on glucose. The final yield on fructose was low for a few fungi. Some of the fungi that grew slower on fructose than on glucose, or produced a lower yield are *Endoconidiophora fimbriata, Monascus purpurea, Stysanus stemonitis* and *Thielaviopsis basicola.*

Margolin (1942) reported a number of fungi to make less growth on fructose than on glucose: *Diplodia macrospora, Phytophthora cactorum, P. erythroseptica, P fagopyri* and *Typhula variabilis. Melanospora zamiae* utilizes fructose poorly (Hawker and Chaudhuri, 1946).

The poorer growth of some fungi on fructose than on glucose could be ascribed to the specific structure of this sugar, or to other factors. Fructose darkens more than glucose or mannose when autoclaved. It is presumed that fructose is altered more by this method of sterilization than are the aldose sugars. Some preliminary experiments, which will not be reported in detail, indicated that an inhibitory substance (s) is formed when fructose is autoclaved with the other constituents of the medium. *Thielaviopsis basicola* was grown on glucose autoclaved with the other constituents of the medium, glucose filtered, fructose autoclaved with the other constituents of the medium, and fructose filtered. The dry weights at 5 days were 72, 92, 7 and 90 mg.; at 9 days 97, 107, 63 and 114 mg., respectively.

On the other hand, Cantino (1951) has reported a species of *Pythiogeton* to utilize fructose rapidly only if it was autoclaved with yeast extract. The effect of autoclaving sugars with the other constituents of the medium may be beneficial for some fungi and inhibitory for others.

Galactose: This hexose is a common component sugar of many oligo- and polysaccharides of animal and plant origin. Galactose is occasionally found free in plants. This sugar occurs in two of the oligosaccharides used in this work, lactose and raffinose. Galactose differs from glucose in the configuration of carbon 4.



D-Galactose

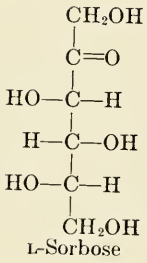
Table 1 shows that *Phytophthora infestans, Schizothecium longicolle,* and *Monascus purpurea* grew poorly on galactose. The rate of initial growth of more than half of the fungi tested was less on galactose than on glucose. The final weight of mycelium produced on galactose was frequently equal to or more than that on glucose. Some fungi utilized galactose rapidly: *Choanephora cucurbitarum, Fusarium culmorum, Gliomastix convoluta* and others.

Other investigators also have reported galactose to be poorly or slowly utilized. Margolin (1942), who used a fixed time of incubation, found 12 of the 21 species studied by him to utilize galactose poorly or slowly. Horr (1936) found *Aspergillus niger and Penicillium glaucum* to grow more slowly on galactose than on glucose; mixtures of these sugars were well utilized. Edgecombe (1938) studied the growth of seven fungi on various media containing galactose. Some of his test fungi grew rapidly on galactose, whereas others showed a prolonged lag period. Unfortunately, the experimental methods used by this investigator make his results difficult to compare with those of others. Hawker (1939) has reported *Melanospora destruens* to be unable to utilize galactose.

Many of the fungi tested grew slowly on galactose at first. This behavior is common among the yeasts. Many yeasts when first placed in contact with galactose fail to utilize this sugar, but, after a time,

which varies with the experimental conditions and the species (Spiegelman, 1945; Sheffner and Lindergren, 1952), galactose is used readily. This is called adaptation, and in the opinion of Spiegelman (1950) is one of the most important properties of organisms. Although studies of adaptation were not made in this work, many instances suggest that the utilization of various sugars is dependent on the formation of adaptive enzymes.

Sorbose: The effect of this sugar on the utilization of other sugars is the subject of the second part of this bulletin. Here, the purpose of our experiments was to determine the rate and amount of growth of the test fungi on sorbose when it was the sole source of sugar-carbon in the medium and to compare the utilization of sorbose with that of other sugars. L-sorbose has been reported to occur in the pectic substances of the passion fruit (Martin and Reuter, 1950). Bertrand (1904) found that this sugar was produced by bacterial (*Acetobacter xylinium* or *A. suboxydans*) oxidation of sorbitol which is a sugar alcohol found in many fruits and prepared commercially by reducing glucose. Until recently L-sorbose was difficult to obtain. Two studies on the utilization of this sugar by filamentous fungi have appeared recently (Barnett and Lilly, 1951; and Oddoux, 1952). Including the information in this bulletin, our information on the utilization of this sugar is now more complete than for many of the common sugars. The structural formula of sorbose is given below.

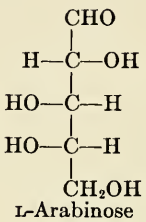


L-Sorbose

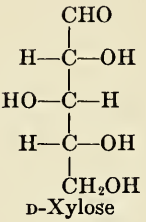
The data in Table 1 indicate that almost without exception the 57 fungi tested grew very slowly, if at all, when first placed in a medium containing sorbose. Some species failed to make a significant amount of growth on sorbose, *e.g., Choanephora cucurbitarum, Sordaria fimicola, Stysanus stemonitis,* and others. Other species made little or no growth for a considerable length of time, but made considerable growth by the time the experiments were terminated, *e.g., Chaetomium globosum, Endoconidiophora virescens, Neocosmopara vasinfecta,* and others.

Other fungi grew slowly on sorbose, but eventually produced approximately the same weight of mycelium as when glucose was used in the medium. Two fungi grew almost as rapidly on sorbose as on glucose, *e.g., Gliomastix convoluta, Fusarium medicaginis. Lenzites saepiaria* appeared to grow more rapidly on sorbose than on glucose.

Arabinose and xylose: The two aldopentoses used in this work will be considered together. These pentoses are widely distributed in nature as constituent sugars of polysaccharides, including hemicelluloses and plant gums. The formulas for these sugars are given below.

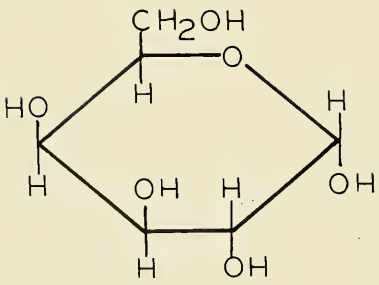


L-Arabinose

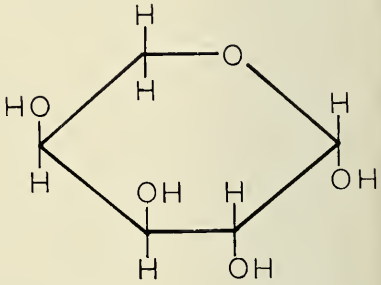


D-Xylose

The configuration of the pyranose ring in *beta*-L-arabinose is identical with that of *alpha*-D-galactose, Pigman and Goepp, (1948). If this configuration has an influence on utilization, it may be expected that the utilization of galactose and arabinose by fungi would show certain similarities.



alpha-D-galactose



beta-L-arabinose

If we consider the initial rate of growth on different sugars to indicate how easily they are utilized, it will be found from the data in Table 1 that both galactose and arabinose are frequently utilized slowly.

Some species used arabinose more rapidly initially than galactose: *Collybia velutipes, Monilinia fructicola,* and *Penicillium spiculisporum.* More often, growth was more rapid on galactose than on arabinose.

Phytophthora infestans made only a trace of growth on either arabinose or xylose. *Schizothecium longicolle*, at the end of 30 days, made only a trace of growth on arabinose, while *Thielaviopsis basicola* did not utilize xylose. With the following exceptions, xylose supported more growth or more rapid initial growth than arabinose: *Endoconidiophora adiposa, E. virescens, Monilina fructicola,* and *Sphaeropsis malorum.*

Utilization of Oligosaccharides

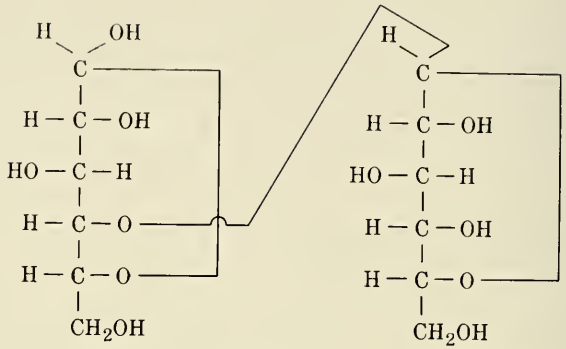
Certain chemical features of the oligosaccharides are pertinent to the discussion to follow. The oligosaccharides are water soluble complex sugars that yield on complete hydrolysis one or more monosaccharides. The monosaccharide residues are united by glycosidic linkages that may differ in type (*alpha, beta,* or mixed) and the carbons involved in the linkage.

Maltose and Cellobiose: These sugars differ only in the type of glycosidic linkage that unites the two glucose residues comprising these sugars. The formulas are given on page 24.

The influence of the type of glycosidic linkage in maltose and cellobiose is reflected in the rate and amount of growth of the test fungi. All of the 57 fungi, with the exception of *Phytohpthora infestans,* utilize cellobiose. The following fungi grew slowly or poorly on maltose: *Cordana* sp., *Monascus purpurea, Monilinia fructicola, Phytophthora infestans,* and *Zygorhynchus vuilleminii.*

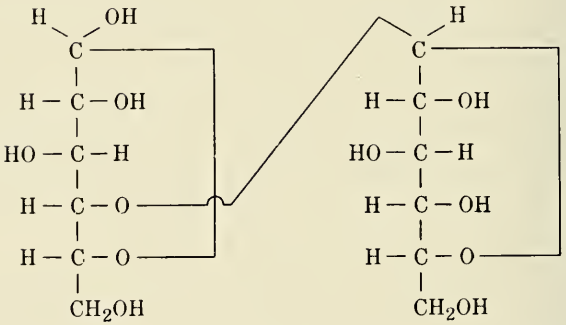
From the results of this work nothing can be said about the pathways of utilization of oligosaccharides when they were utilized *well.* In those instances where oligosaccharides were utilized *poorly,* and the component sugars were well utilized, certain negative conclusions may be reached. On this basis, it may be assumed that those species that failed to utilize maltose, or utilized this sugar slowly, produced only a trace of maltase. Had these species been able to hydrolyze maltose, glucose would have been the only sugar produced. All of the fungi that failed to utilize maltose well made good growth on glucose. It also may be concluded that these species lack any efficient alternative pathway for utilizing maltose under these experimental conditions.

Hawker and Chaudhuri (1946) correlated the rate and amount of growth of *Podospora* sp., *Chaetomium cochloides,* and *Pyronema confluens* on sucrose with the amount of sucrase found in the mycelium. *Podospora* sp., which made only a trace of growth on sucrose, had only a trace of sucrase activity, whereas mycelium of *Pyronema confluens* was active in this respect. *Chaetomium cochloides* mycelium was intermediate in ability to hydrolyze sucrose. The rate of growth of these



Maltose

(4-D-glucose *alpha*-D-glucopyranoside)



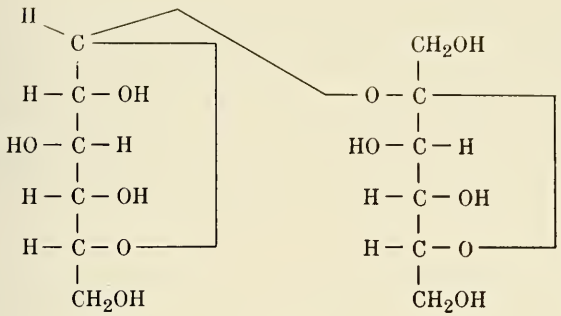
Cellobiose

(4-D-glucose *beta*-D-glucopyranoside)

three species correlated very well with the rate of disappearance of surcrose from the medium and the activity of the mycelium in hydrolyzing sucrose.

About one-fourth of the 57 fungi tested utilized cellobiose nearly as rapidly as glucose, *e.g., Gliomastix convoluta, Schizothecium longicolle,* and *Sordaria fimicola.* The initial rate of growth of more than half of the fungi on cellobiose was less than when glucose was the sugar used. This behavior is consistent with the view that cellobiose is hydrolyzed before utilization by an adaptive enzyme.

Sucrose: This disaccharide yields glucose and fructose on hydrolysis. Sucrose is rapidly hydrolized under acid conditions. The formula for this non-reducing sugar is given opposite.



Sucrose

(1-*alpha*-D-glycopyranose *beta*-D-fructofuranoside)

Most of the fungi tested utilized sucrose. The initial rate of growth of a few fungi was slower on sucrose than on glucose; *e.g., Alternaria solani, Chaetomium globosum,* and *Thielaviopsis basicola.* This work and that of Margolin (1942) also indicate that a considerable number of fungi are either unable to utilize sucrose, or do so with

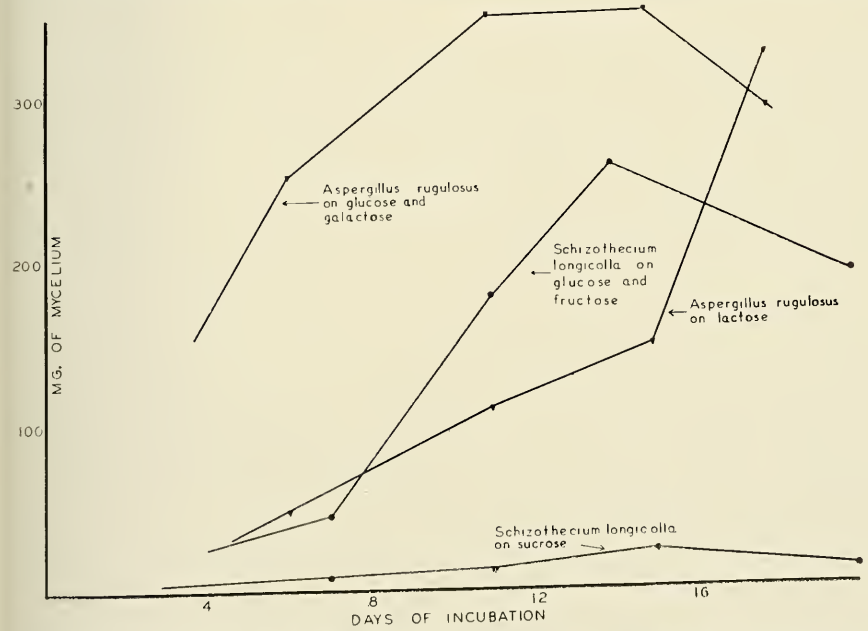


FIGURE 1. GROWTH of Aspergillus rugulosus on lactose and an equivalent amount of its hydrolytic products (glucose and galactose). Growth of Schizothecium longicolle on sucrose and its hydrolytic products (glucose and fructose). Note that this fungus grows much better on invert sugar than sucrose.

extreme slowness. Fungi that utilized sucrose poorly include *Monascus purpurea, Mucor ramannianus,* and *Sordaria fimicola.*

A comparative study of the rate and amount of growth of six species unable to utilize sucrose was made on sucrose and on equivalent amounts of glucose and fructose. These results are assembled in Table 2. From these data it may be concluded that fungi unable to utilize sucrose synthesize little or no sucrase under our conditions. Some of these data are shown graphically in Figure 1.

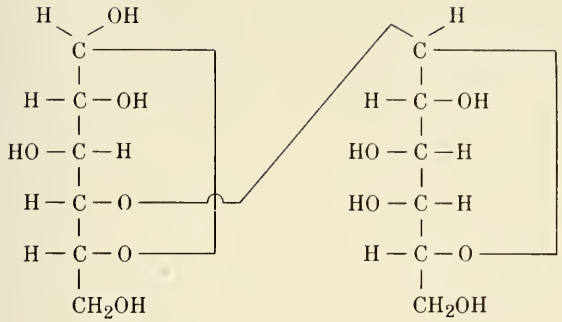
TABLE 2. THE AMOUNT OF GROWTH IN MG. OF 6 FUNGI ON SUCROSE AND ON AN EQUIVALENT AMOUNT OF GLUCOSE AND FRUCTOSE

Species	Days of Incubation	23.7 g. sucrose per liter	12.5 g. each of glucose and fructose
Choanephora cucurbitarum	4	11	41
	6	14	65
	8	13	65
Cordana sp.	18	12	83
	25	9	136
	30	7	195
Mucor ramannianus	4	15	89
	6	14	103
	11	11	207
	15	24	167
Polyporus albellus	20	5	22
	27	4	49
	34	3	89
	40	7	157
	50	9	190
Schizothecium longicolle	7	9	46
	11	11	177
	15	24	258
	20	12	190
Sordaria fimicola	4	11	121
	6	18	192
	11	11	199
	15	24	167

Were these fungi able to hydrolyze sucrose, they would have grown on the mixture of glucose and fructose so produced. This argument does not prove that fungi that utilize sucrose hydrolyze it before utilization, although they may well do so.

Lactose: This sugar is found in the milk of mammals; it has not been reported from plants. The formula is given opposite.

In a way, results of this investigation confirm the belief that lactose is a poor source of carbon for fungi. This is certainly true for many fungi, especially if the time of incubation is short. It also is true that many fungi produce about as much mycelium on lactose



Lactose

(4-D-glucose *beta*-D-galactopyranoside)

as on glucose, provided that sufficient time is given. Most of the fungi tested utilized lactose slowly. Two species, *Colletotrichum lindemuthianum* and *Gliomastix convovluta,* utilized lactose rapidly. Examples of fungi that did not utilize lactose or did so with extreme slowness are *Endoconidiophora fimbriata, Choanephora cucurbitarum,* and *Monilinia fructicola.*

Separate experiments were made to compare the growth of some of the fungi on lactose and its hydrolytic products. These results are shown in Table 3 and may be interpreted as indicating that fungi failing to grow on lactose are unable to hydrolyze this sugar. Since the fungi that grew slowly on lactose grew much faster on glucose and galactose (excepting perhaps *Melanconium fuligineum),* this probably means that the synthesis of lactase is a limiting factor in the rate of growth of these fungi.

A comparison of the data in Tables 1 and 3 indicates that some fungi make more growth on a mixture of glucose and galactose than on either sugar alone, *e.g., Endoconidiophora fimbriata, Penicillium expansum, Fusarium niveum,* and *Sphaeropsis malorum.* On the other hand, *Melanconium fuligineum* grew less well on a mixture of glucose and galactose up to the 12th day of incubation than on either of these sugars alone. *Schizothecium longicolle* grew about equally well on the mixture of glucose and galactose as on glucose alone; this fungus makes poor growth of galactose alone. Horr (1936) and Steinberg (1939) have shown that it is not always possible to predict the amount of mycelium that will be produced on mixtures of sugars from data on the amount of growth produced on individual sugars. Hawker (1947) showed that glucose, fructose, and mixtures of these two sugars are not equivalent to sucrose for growth and perithecial formation by *Melanospora destruens.*

TABLE 3. THE AMOUNT OF GROWTH IN MG. OF 20 FUNGI ON LACTOSE AND ON AN EQUIVALENT AMOUNT OF GLUCOSE AND GALACTOSE

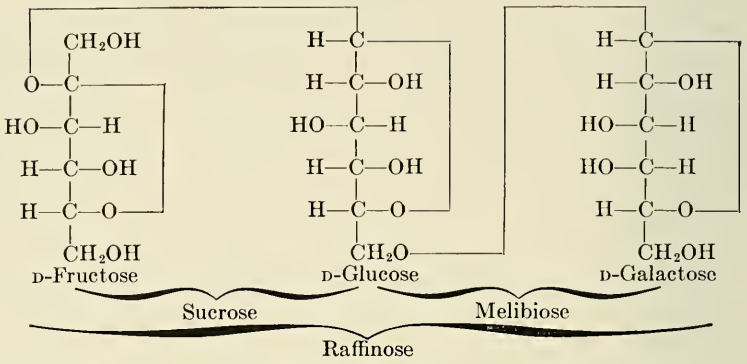
Species	Days of incubation	25 g. lacotse monohydrate/l.	12.5 g. each of glucose and galactose
Aspergillus elegans	3	7	146
	8	21	187
	11	32	181
	17	78	154
Aspergillus rugulosus	6	49	253
	11	110	351
	15	148	353
	18	326	294
Chaetomium globosum	4	2	20
	7	9	76
	10	22	251
	15	100	348
Choanephora cucurbitarum	3	8	25
	5	5	65
	7	8	77
	13	12	81
Endoconidiophora fimbriata	3	6	19
	5	8	96
	7	8	87
	12	10	156
Endothia parasitica	5	6	31
	8	11	55
	12	22	73
	20	24	62
Fusarium culmorum	3	11	21
	8	33	89
	12	44	109
	17	63	131
Fusarium conglutinans	5	65	254
	10	186	279
	12	190	283
	15	181	279
Fusarium lycopersici	3	35	142
	7	158	296
	12	181	240
	15	182	201
Fusarium medicaginis	3	30	79
	5	167	244
	7	178	213
Fusarium niveum	3	7	49
	7	26	231
	12	23	251
	19	30	225
Fusarium tracheiphilum	3	23	61
	4	33	181
	6	100	245
	10	110	184

TABLE 3 (CONTINUED)

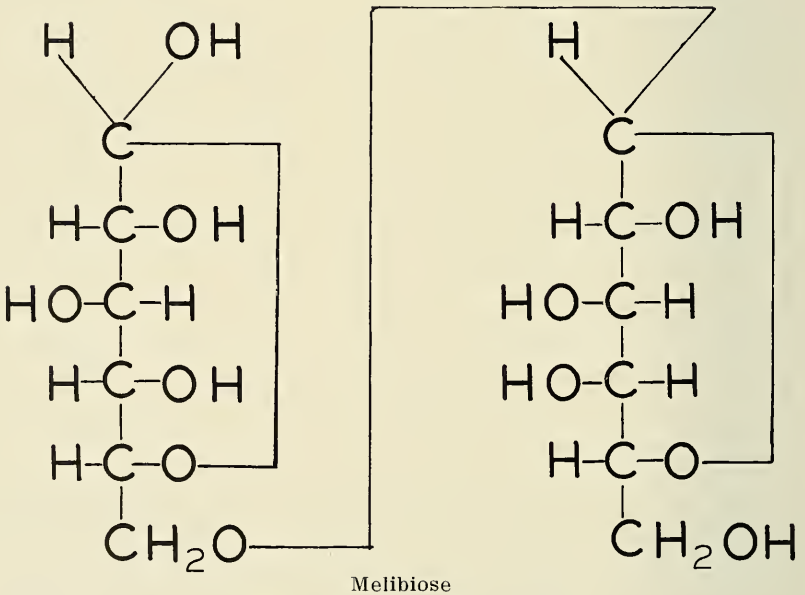
Species	Days of incubation	25 g lactose monohydrate/l.	12.5 g. each of glucose and galactose
Glomerella cingulata	3	10	163
	5	60	268
	7	61	315
	13	190	252
Melanconium fuligineum	6	4	32
	8	14	36
	12	12	54
	17	14	220
Phycomyces blakesleeanus	3	3	43
	7	3	147
	11	5	168
Penicillium chrysogenum	3	8	115
	5	19	257
	12	167	177
	17	157	127
Penicillium expansum	3	10	140
	5	17	191
	11	33	239
	17	49	192
Penicillium spiculisporum	6	10	176
	8	17	243
	14	37	227
	18	46	230
Schizothecium longicolle	6	2	35
	10	5	135
	12	9	189
	17	22	213
Sphaeropsis malorum	4	7	60
	6	13	112
	12	33	306
	20	56	283

Raffinose: This oligosaccharide is found in many plants including sugar beets, cotton, and Eucalyptus. Complete hydrolysis yields equal amounts of glucose, fructose, and galactose. The raffinose molecule contains the disaccharide structures of sucrose and melibiose. Melibiose is prepared from raffinose by allowing *Saccharomyces cerevisiae* to hydrolyze away and utilize the fructose moiety (Hudson and Harding, 1915; Fletcher *et al.*, 1952). The formulas for raffinose and melibiose are shown on page 30.

About one-third of the fungi tested made poor or slow growth on raffinose. Some of the fungi utilized raffinose well, *e.g., Fusarium medicaginis, Phoma betae,* and *Sphaeropsis malorum.* Other fungi grew slowly on raffinose but eventually produced as much mycelium



1-[6-(*alpha*-galactopyranosyl)-*alpha*-D-glucopyranose] *beta*-D-fructofuranoside



Melibiose

6-glucose *alpha*-D-galactopyranoside

as on glucose, *e.g., Alternaria solani, Helminthosporium sativum,* and *Thielaviopsis basicola.* A number of fungi apparently were unable to utilize raffinose, *e.g., Choanephora cucurbitarum, Endoconidiophora fimbriata, Monascus purpurea, Mucor ramannianus, Phytophthora infestans,* and *Sordaria fimicola.* Other species utilized raffinose only

after long periods of incubation, *e.g., Cordana* sp., *Lenzites saepiaria,* and *Phycomyces blakesleeanus.* Still other species made from one-third to one-half the amount of growth that occurred on glucose, *e.g., Aspergillus clavatus, Endoconidiophora fagacearum,* and *Gliomastix convoluta.* This latter behavior suggests that either the fructose or galactose moiety is being rather rapidly cleaved from the raffinose molecule, which would leave melibiose or sucrose, respectively. This simple theory is not adequate to explain some of the results obtained in the next experiment.

A limited number of fungi that utilized raffinose slowly or poorly were reinvestigated on the component sugars that occur in the raffinose molecule. These data are given in Table 4.

Table 1 shows that *Endoconidiophora fimbriata* utilized fructose and galactose rather slowly, but produced a normal amount of mycelium on these sugars. Only a trace of growth occurred on raffinose. Sucrose also was used well. From Table 4 it is seen that melibiose is not used by this species. On the basis of this evidence it appears that *E. fimbriata* is unable to utilize raffinose because it is unable to hydrolyze the raffinose molecule.

From the data on raffinose alone, *Gliomastix convoluta* appears to utilize about one-third of the available carbon; yield on raffinose at 9 days, 98 mg.; yield on the three component sugars, 270 mg. Not only are simple component sugars well utilized, but sucrose and melibiose are utilized, although melibiose is used more slowly than sucrose. If it were assumed that *G. convoluta* hydrolyzed off galactose, the sucrose would be utilized. A mixture of sucrose and galactose is utilized even more efficiently than a mixture of the three monosaccharides found in raffinose, *i.e.,* the yield from sucrose and galactose at 9 days was 319 mg. The mixture of melibiose and fructose was used with slightly less efficiency than the mixture of sucrose and galactose. Since all of the mono- and disaccharides occurring in the raffinose molecule and mixtures of these sugars are utilized well there remains an unanswered question: Why should *G. convoluta* make about one-third the expected amount of growth on raffinose alone? Certain of these results are illustrated in Figure 2.

The results with *Sordaria fimicola* are equally interesting. This species utilizes glucose, fructose, and galactose, but utilizes neither sucrose nor raffinose (data on single sugars, Table 1). From Table 4 it is seen that melibiose alone is not utilized, whereas a mixture of the three simple component sugars of the raffinose molecule is well utilized. The yield at 15 days on sucrose alone was 15 mg., on galactose alone 83 mg. The yield on the mixture of sucrose and galactose was 200 mg., whereas the calculated yield was only 98 mg. Although proof is

TABLE 4. THE AMOUNT OF GROWTH IN MG. OF 10 FUNGI ON RAFFINOSE AND ITS COMPONENT SUGARS

Species	Days of incubation	27.6 mg. raffinose pentahydrate	8.33 g. each of glucose, fructose and galactose	8.33 g. fructose	8.33 g. galactose	15.8 g. sucrose	15.8 g. sucrose and 8.33 g. galactose	16.67 g. melibiose	16.67 g. melibiose and 8.33 g. fructose
Choanephora cucurbitarum	3	16	38	26	13	24	29	8	47
	5	19	48	32	68	11	73	5	57
	12	11	81	33	57	14	87	12	94
Cordana sp.	10	10	39	22	25	9	28	4	25
	15	4	110	42	44	7	67	4	57
	20	3	164	55	68	7	115	6	112
	25	5	169	50	56	7	92	15	114
Endoconidiophora fimbriata	4	12	51	46	30	71	73	5	51
	8	13	124	64	96	121	130	7	64
	12	24	161	63	73	145	181	14	82
	16	16	146	61	60	127	195	14	73
Gliomastix convoluta	4	46	152	56	67	81	98	36	124
	6	87	248	106	128	204	288	72	225
	9	98	270	79	86	205	319	103	273
	15	81	214	63	64	161	250	165	275
Mucor ramannianus	4	10	71	47	34	9	55	2	52
	6	11	87	84	72	11	91	2	102
	9	11	129	84	63	11	102	7	108
	15	8	204	72	66	5	96	4	88
Neocosmospora vasinfecta	4	43	58	100	75	88	142	31	72
	7	124	135	101	97	164	158	36	154
	9	165	101	99		146	180	78	141
	15		121	64	73	117	149	112	112
Phycomyces blakesleeanus	6	11	94	89	49	82	128	3	105
	9	17	148	90	73	130	128	4	123
	12	9		73	68	139	152	2	105
	18	16		77	64	132	125	2	94
Schizothecium longicolle	6	5	53	33	9	7	17	7	40
	9	8	120	75	8	9	14	7	107
	15	15	210	108	4	8	15	11	139
	20	21	226	59	5	6	11	16	115
Sordaria fimicola	5	7	143	112	22	11	69	8	153
	8	10	260	108	41	16	138	10	179
	11	10	258	81	76	16	168	8	137
	15	9	230	71	83	15	200	11	146
Thielaviopsis basicola	4	20	115	55	46	121	175	9	100
	7	16	166	89	91	171	203	10	171
	11	16	205	73	76	189	241	13	132
	18	18	170	61	90	150	202	10	92

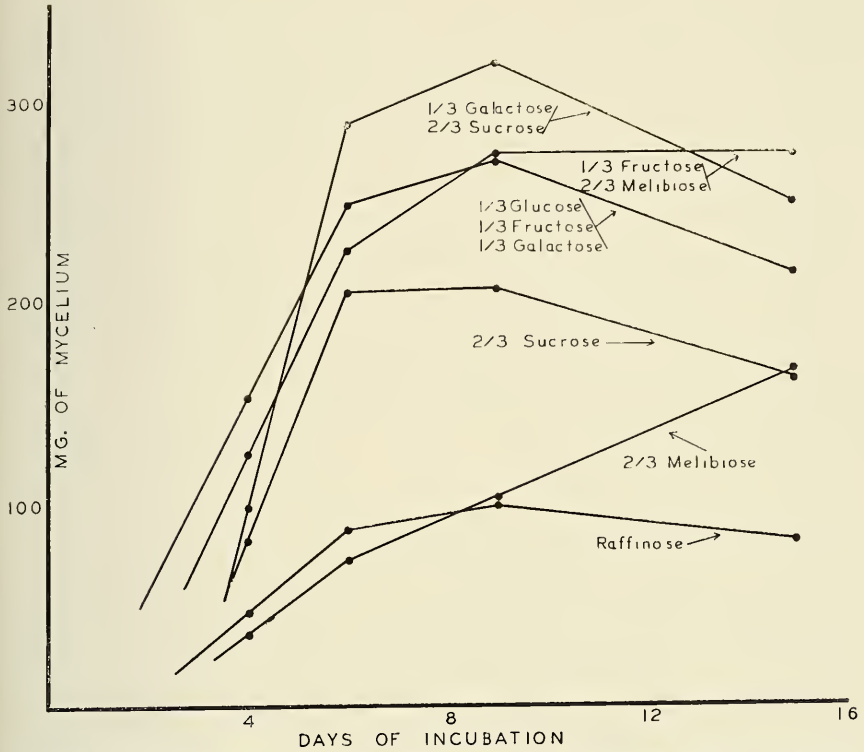


FIGURE 2. GROWTH of Gliomastix convoluta on raffinose and on the component sugars of raffinose. Note that this fungus apparently utilizes the component sugars of raffinose better than raffinose alone.

lacking, it appears probable that sucrose is utilized when galactose is present in the medium. A less striking increase in yield was found for the combination melibiose and fructose. Some of the data for this experiment are illustrated in Figure 3. Fructose is utilized rapidly by *S. fimicola* and this influence is carried over in the utilization of the combination of fructose and melibiose. Galactose is utilized slowly. This is reflected to some degree in the rate of growth on the combination of sucrose and galactose.

Schizothecium longicolle is like *Sordaria fimicola* in that it is unable to utilize raffinose, sucrose, or melibiose, and unlike in that it utilizes galactose slowly. From the data in Table 1 it might be concluded that the initial growth of *S. longicolle* on galactose is due to some impurity in the sugar. The combination of sucrose and galactose, neither of which are used separately, was not used. However, growth was somewhat better on the combination of melibiose and fructose than on fructose alone.

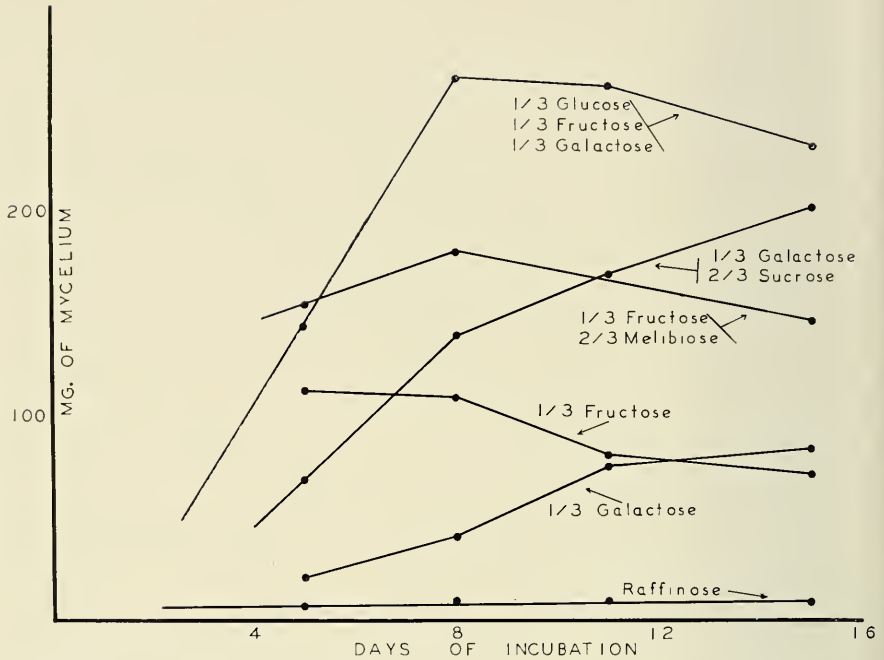


FIGURE 3. GROWTH of Sordaria fimicola on raffinose and some of its component sugars. Note the influence of fructose on the utilization of melibiose and the influence of galactose on the utilization of sucrose. Neither melibiose nor sucrose were appreciably utilized in the absence of fructose or galactose (Table 4).

The data for *Thielaviopsis basicola* illustrates the unexpected increase in the amount of growth that occurs when certain fungi are grown on specific mixtures of sugars. This species makes only a trace of growth on melibiose when this sugar only is present. Growth is rapid on fructose. Growth is rapid on a mixture of fructose and melibiose, and the amount of growth on this combination of sugars makes it highly probable that melibiose is utilized in the presence of fructose. These data and the control (one-third each of glucose, fructose, and galactose) are illustrated in Figure 4.

Cordana sp. utilizes a mixture of glucose, fructose, and galactose with much greater efficiency than mixtures of sucrose-galactose and melibiose-fructose. However, neither sucrose nor melibiose are used appreciably when they are the sole sources of sugar-carbon. These disaccharides appear to be utilized, to some extent at least, when galactose and fructose respectively are present.

It appears self-evident that the fungi that fail to utilize raffinose lack the necessary enzymes to hydrolyze this sugar. The results with

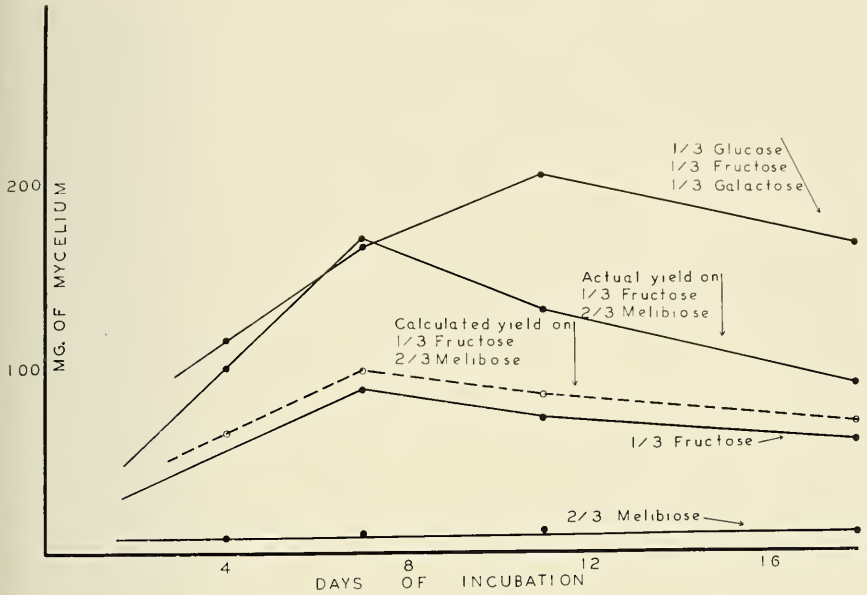


FIGURE 4. GROWTH of Thielaviopsis basicola on some of the component sugars of raffinose. Note the melibiose was not appreciably utilized unless fructose was also present. The actual yield greatly exceeded the calculated yield for the mixture of fructose and melibiose.

the single and mixed sugars derivable from raffinose by hydrolysis indicate, for some species, that a "non-utilizable" sugar may be utilized if a readily utilizable sugar also is present. These results are in agreement with some experiments of Norkrans (1950) who reported *Tricholoma flavobrunneum* to utilize cellulose provided some glucose ("start-glucose") was present initially. Since mixed carbohydrate sources are encountered almost exclusively in nature, much more experimental work is needed before we can properly assess the ability of fungi to utilize carbohydrates.

Discussion

One general conclusion reached is that, with one or two exceptions, these 57 fungi are able to utilize most of the 12 sugars for growth. A few fungi grew rapidly on all of the 12 sugars (*Fusarium lycopersici* and *Glomerella cingulata*), but even these fungi grew at a significantly slower rate on some sugars during the early days of incubation. Most of the fungi grew slowly on at least one of the 12 sugars, and many grew slowly on two or more sugars. A few species apparently did not utilize certain sugars under the conditions used in this work. Had

the time of incubation been restricted to that required for maximum yield on the best sugar, it would have been concluded that many more sugars were poorly utilized. Had the time of incubation been extended, it is possible that some fungi would have become adapted to utilize certain sugars not utilized under our conditions.

It was difficult to determine whether some of the fungi utilized certain sugars. Initially, these species either made little growth which soon ceased, or continued to grow very slowly over a long period of incubation. The first type of behavior may be due either to utilization of impurities in the sugars used, or to sugar introduced with the inoculum. As examples of this behavior see the data in Table 1 for *Zygorhynchus vuilleminii* on sorbose and *Sordaria fimicola* on sucrose. Second, some fungi made only a trace of growth during the initial period of incubation, but as the period of incubation was prolonged the fungi began to grow, slowly at first and then more rapidly. This probably means that adaptive enzymes have to be formed before the sugar in question is utilized. Many examples of this type of growth may be found in Table 1.

The main purpose of this work was to determine how well the various sugars were used for growth, not to explore the possible pathways of sugar utilization. However, certain conclusions may be reached from the data available in Tables 1 to 4. Two general theories of oligosaccharide utilization may be considered. (1). The oligosaccharide is hydrolyzed to its component sugars, which are then utilized. The name of Emil Fischer is associated with the view that failure to utilize an oligosaccharide is due to the failure of the organism to synthesize the necessary hydrolytic enzymes. (2). The oligosaccharides are utilized by pathways that do not require hydrolysis before utilization. Richard Willstäter was led to the "direct" theory of utilization of quantitative studies of the amount of oligosaccharides fermented and the enzyme content of the cells. Both of these theories were developed from work with yeasts. Hestrin (1948) has reviewed the evidence supporting these theories. Evidence obtained in this work, insofar as the poorly utilized oligosaccharides are concerned, supports the Fischer hypothesis.

No evidence was obtained that would relate sugar utilization to taxonomic position or natural habitat of the fungus. Perhaps this is because an insufficient number of species and sugars were tested. Three or more species of *Fusarium*, *Endoconidiophora*, *Aspergillus* and *Penicillium* were used, yet the species within these genera were not uniform in their ability to utilize the 12 sugars. Note that *Fusarium niveum* utilized lactose slowly, *Endoconidiophora adiposa* utilized sorbose rapidly after a short period of slow initial growth. *Penicillium chrysogenum* utilized lactose more efficiently than *P. expansum* or *P. spiculisporum*.

Aspergillus rugulosus utilized sorbose to better advantage than *A. clavatus* or *A. elegans.*

We may conclude that the ability or inability to utilize a certain sugar under a given set of conditions is an inherent quality of the species and perhaps in some cases, even an isolate within the species. It is true, however, that some similarities between closely related species do exist. Species of *Fusarium, Aspergillus,* and *Penicillium,* for example, are known to possess a rather complete complement of enzymes involved in nutrition. They are known to utilize a wide variety of nitrogen and carbon sources and are mostly autotrophic for vitamins. Other species, or isolates, lack certain enzymes necessary for the utilization of certain sugars.

It also is interesting to note that all of the plant pathogenic species listed in Table 1, with the exception of *Choanephora cucurbitarum,* are able to utilize sucrose readily. Although sucrose is one of the most common sugars found in plants, it is possible that the flowers and young fruit of the cucurbits and certain other plants attacked by *C. cucurbituarm* contain an additional sugar which is more readily utilized.

The isolates of *Phycomyces blakesleeanus* and *Mucor ramannianus* were the same ones used by Margolin (1942) in sugar utilization studies ten years ago. No change in the pattern of sugars utilized was found. The isolate of *Sordaria fimicola* used by Margolin was obtained from Baarn; the culture used in this investigation was isolated locally from dung. Both isolates had the same pattern of sugar utilization.

The results when certain fungi were grown upon media containing two or more sugars are extremely suggestive. Sugars not appreciably utilized when present in the medium singly may be utilized rapidly if an easily utilizable sugar also is present (Table 4). Insufficient attention has been given to this type of behavior and its possible significance. Substrates containing mixed carbohydrate sources are the rule in nature. To answer the question "Which sugars are utilized by a given species?" it appears to be necessary to test mixtures as well as individual sugars. It is possible that the other constituents of the medium, such as the nitrogen source, or some environmental condition such as the temperature, may have a profound effect on the utilization of carbohydrates.

Part II
THE EFFECT OF SORBOSE ON THE UTILIZATION OF CERTAIN OTHER SUGARS

The data presented in Part I show that species of fungi vary widely in the utilization of sugars when used singly. Growth of fungi on media containing mixtures of sugars has received little critical attention. There are only a few reports (Horr, 1936; Margolin, 1942) of the effects of the presence of one sugar on the utilization of another. The effect may be stimulatory, inhibitory, or none. For example, galactose alone is a relatively poor source of carbon for *Aspergillus niger*. Horr (1936) has shown that the presence of glucose increased the utilization of galactose by this fungus. It made 42.4 mg. of dry weight on 18 g. of galactose and 154.6 mg. on 2 g. of glucose. In a mixture of 18 g. galactose and 2 g. glucose the dry weight was 577.4 mg. Steinberg (1939) showed that the effects of mixing mannitol and lactose were similar, but a mixture of glycerol and galactose gave less than the calculated yield.

On the other hand, it has been shown that the presence of sorbose inhibits the utilization of glucose, sucrose, and maltose by certain fungi (Barnett and Lilly, 1951). Other species showed no inhibition. Tatum *et al.* (1949) also have noted the inhibitory effect of sorbose on the growth of *Neurospora* spp. and *Syncephalastrum racemosum*. These investigators used a medium containing sorbose to restrict the extension of mycelium in mutation studies. The effect of mixed carbon sources on the growth of *Phycomyces blakesleeanus* was believed to be purely additive (Margolin, 1942). No one has offered a satisfactory explanation of how one sugar affects the utilization of another.

Much of the data and discussion in Part II will concern the growth of 55 species of fungi in media containing single sugars (glucose and maltose) and mixtures of sorbose with glucose and maltose. Even though sorbose is rarely found in nature, it was selected to be used because it is utilized poorly by many species and because of the degree of its inhibitory action in combination with other sugars. This investigation should be considered only as a portion of a broader and much needed study of the effects of other mixed carbon sources in various combinations on the growth of a large number of fungi. Such a study is essential to the understanding of the growth of fungi in nature.

A brief report of the essential features of the inhibitory effects of sorbose on 12 species of fungi included herein has been published (Barnett and Lilly, 1951). The present report extends the list of species to 55 and gives additional data that aid in understanding the principles involved.

Materials and Methods

The basal medium and the cultural conditions used in experiments on the effects of sorbose were the same as those described in Part I except in sugar concentrations used. In all of the experiments described below, the amount of each sugar was 20 g. per liter unless otherwise stated. For example, in Table 5 each sugar was 20 g. per liter except when the total concentration was compared by use of 40 g. of glucose. Asparagine (2 g. per liter) served as the source of nitrogen except when otherwise stated. Yeast extract was used in early experiments, some of which are reported herein. Special methods, media, and equipment are described under the particular experiments. The species of test fungi were chosen at random, mostly from the stock culture collection at hand. No attempt was made in this study to compare different isolates of the same species. Temperature of incubation was 25° C. unless otherwise stated.

Experimental Results

Growth of fungi in single sugars and in mixtures with sorbose. Table 5 shows the growth (in mg. dry weight) of 55 species of fungi in liquid media varying in kind and amount of sugar. In most cases data were taken after two different incubation periods. The first harvest was usually made before maximum growth was attained in glucose or maltose medium, and the second harvest was made near the time of maximum growth on these sugars. Differences in growth are greater during this early period than later. The conclusions based on these data are *applicable only for these conditions* and in some cases would likely be different if the incubation period was greatly extended or various changes made in the media.

Certain variations in data from duplicate experiments conducted at different times cannot be explained, but the same principles were illustrated by all repetitions of an experiment.

The data in Table 5 show that these fungi fall roughly into three groups based on their response to sorbose either alone or when mixed with maltose. Group A contains species that utilize sorbose well, even though slower than glucose or maltose is utilized. Group B is composed of fungi that utilize sorbose slowly or not at all, making less than half the

TABLE 5. EFFECT OF SORBOSE ALONE AND MIXTURES WITH GLUCOSE AND MALTOSE ON GROWTH IN MG. OF 55 FUNGI. SPECIES ARE LISTED BY 3 GROUPS: A. THOSE WHICH UTILIZE SORBOSE WELL; B. THOSE WHICH UTILIZE SORBOSE POORLY BUT ARE NOT GREATLY INHIBITED BY ITS PRESENCE; AND C. THOSE WHICH ARE GREATLY INHIBITED BY THE PRESENCE OF SORBOSE. T = TRACE OF GROWTH, LESS THAN 20 MG.

Fungi	days	glucose	glucose sorbose	maltose	sorbose maltose	sorbose	glucose 2X
Group A							
Aspergillus niger	6	239	284	207	326	86	316
	14	220	292	192	274	176	298
Aspergillus rugulosus	6	231	318	299	306	33	327
	13	239	439	200	220	189	448
Botrytis cinerea	5	136	226	190	172	45	343
	9	182	266	210	360	198	379
Endoconidiophora adiposa	7	183	268	158	247	240	169
Fusarium conglutinans	4	185	252	200	250	64	320
	7	226	373	226	356	217	374
Fusarium culmorum	4	79	103	109	100	26	92
	7	120	147	135	191	84	144
Fusarium lycopersici	6	226	348	248	350	213	
	8	210	385	229	292	241	412
Fusarium medicaginis	4	137	124	195	140	61	262
	7	167	212	186	310	175	271
Fusarium nivale	5	95	121	171	117	26	
	9	131	164	147	225	72	126
Fusarium niveum	4	167	155	152	177	74	233
	7	181	261	227	246	195	252
Fusarium trachiphilum	4	209	218	205	247	183	264
	7	232	329	211	355	204	298
Gliomastix convoluta	4	144	185	177	225	48	
	9	240	380	227	386	215	
Glomerella cingulata	6	227	320	211	199	78	
	8	182	306	159	298	126	468
Lenzites sacpiaria	13	20	77	91	117	45	296
	21	100	165	149	159	90	274
Penicillium chrysogenum	3	159	166	69	89	41	195
	9	166	375	171	309	138	334
Penicillium expansum	5	177	241	153	211	179	214
Group B							
Aspergillus elegans	5	160	124	107	242	32	230
	8	181	226	145	217	39	234
Chaetomium convoluta	6	55	104	49	85	T	72
	12	163	122	183	148	T	213
Choanephora cucurbitarum	5	75	104	44	45	T	100
	9	65	122	95	101	4	93

TABLE 5 (CONTINUED)

Fungi	days	glucose	glucose sorbose	maltose	sorbose maltose	sorbose	glucose 2X
Colletotrichum lindemuthianum	14	22	14	80	10	T	106
	54	108	194	115	170	20	220
Colletotrichum phomoides	6	159	106	137	12	2	
	13	164	302	151	291	13	
Collybia velutipes	14	193	78	133	59	T	325
	19	116	173	156	163	14	191
Cordyceps militaris	5	134	240	143	247	T	188
	8	139	193	149	224	T	182
Dendrophoma obscurans	8	56	130	58	59	10	
	10			110	65	T	
Endoconidiophora virescens	7	106	149	137	113	9	238
Endothia parasitica	6	67	123	99	160	17	113
	9	201	281	191	162	11	167
Helminthosporium sativum	4	144	185	177	225	48	
	9	240	380	227	386	215	
Lenzites trabea	7	20	46	26	68	20	82
	11	121	145	65	128	21	105
Melanconium fuligineum	14	215	215	167	109	28	323
	19	145	268	151	165	45	364
Monilinia fructicola	7	146	141	26	47	7	173
	10	173	161	27	115	31	284
Mucor ramannianus	6	98	150	84	122	15	158
	11	186	186	126	156	10	212
Neocosmospora vasinfecta	5	230	266	232	257	56	369
	9	222	255	230	200	56	330
Penicillium spiculisporum	10	135	325	216	125	18	284
	16	108	229	157	235	46	254
Phoma betae	6	61	29	114	42	T	84
	12	148	138	163	192	31	275
Phycomyces blakesleeanus	5	154	146	43	68	T	180
	8	167	219	180	233	T	194
Polyporus albellus	16	68	39	83	16	T	105
	21	97	191	94	80	T	153
Polyporus versicolor	5	24	36	18	42	12	22
	9	152	232	124	192	47	222
Schizophyllum commune	9	200	156	94	51	T	342
	12	257	310	234	221	T	422
Stysanus stemonitis	5	93	110	58	30	T	120
	12	136	151	147	90	T	201
Zygorhynchus vuilleminii	5	157	147	38	92	22	216
	10	130	116	43	57	21	213

TABLE 5 (CONTINUED)

Fungi	days	glucose	glucose sorbose	maltose	sorbose maltose	sorbose	glucose 2X
Group C							
Alternaria solani	8	99	81	140	33	20	180
Alternaria tomato	4	30	17	51	14	6	
	9	217	118	221	87	43	
Aspergillus clavatus	6	111	105	91	24	T	127
	9	183	166	173	44	T	215
Chaetomium globosum	6	231	113	254	15	T	364
	12	373	292	364	25	T	375
Endoconidiophora fagacearum	10	91	24	54	10	T	136
Endoconidiophora fimbriata	5	113	T	84	T	T	107
	9	188	T	126	T	T	192
Ophiobolus graminis	9	95	T	55	T	T	87
	12	167	41	139	18	T	246
Rhizoctonia solani	9	341	210	337	7	T	
	13	344	519	299	20	T	
Schizothecium longicolle	8	68	36	57	T	T	83
	29	130	131	128	31	T	192
Sclerotinia sclerotiorum	9	94	20	68	T	T	
	13	187	64	164	T	T	
Sordaria fimicola	7	232	64	224	20	T	
Sphaeropsis malorum	5	153	140	166	61	T	240
Thielaviopsis basicola	7	91	T	109	T	T	143

growth on sorbose than on glucose or maltose, but which are not greatly inhibted by sorbose in mixtures with maltose or glucose. The species of Group C utilize sorbose very slowly or not at all and growth on maltose and often on glucose medium is greatly inhibited by an equal amount of sorbose. This inhibition is greatest during early growth.

Growth of most of the species on sorbose media may be compared in Tables 1 and 5. It must be remembered, however, that the purposes of these two separate investigations were different. The results presented in Table 1 cover a longer period of incubation than those in Table 5, where differences in early growth due to sorbose were of primary interest.

With the exception of a few species the test fungi fall rather closely into one of the three groups. However, there might be some question whether *Colletotrichum lindemuthianum, Collybia velutipes, Phoma betae, Polyporus albellus,* and *Schizophyllum commune* should be placed in Group B or C on the basis of the shorter period of incubation. Since

a slightly longer growth period showed increased growth rate on the maltose-sorbose medium, these species are placed in Group B. Similarly, *Fusarium nivale* might be placed in Group B, but because the weight in sorbose medium at the 9-day period is greater than half the amount in glucose, it is placed in Group A. Reference to Table 1 will give the amount of growth of these species after longer periods of incubation.

The addition of sorbose to an equal amount of maltose results in a greater increase in rate of growth of a few fungi than can be accounted for by the sum of the weights on the two sugars alone; *e.g., Aspergillus elegans* and *Cordyceps militaris* (Table 5).

Oddoux (1952) reported that 50 of the 95 species of Homobasidiomycetes tested failed to make appreciable growth on sorbose medium. When 19 species were used as test fungi the presence of sorbose did not inhibit growth in a glucose medium.

It is evident from Table 5 that the inhibition due to sorbose is greater in the presence of maltose than with glucose. This is illustrated by *Sphaeropsis malorum, Aspergillus clavatus, Schizothecium longicolle,* and *Chaetomium globosum*. Some data not included in Table 1 show that the inhibition also is greater in the presence of sucrose than with glucose. The dry weight of mycelium of *C. globosum* after 12 days (in the same experiments reported in Table 5) was 371 mg. in sucrose and 61 mg. in sucrose-sorbose medium. *Sphaeropsis malorum* made 288 mg. growth after 6 days in sucrose and 87 mg. in sucrose-sorbose medium.

Since sorbose is obtained from sorbitol it was suggested that this sugar alcohol might have the same toxic effect on fungi. When amounts of growth in maltose, maltose-sorbose, and maltose-sorbitol media were compared, it was revealed that sorbitol had no inhibitory effect on the utilization of maltose, even though sorbitol alone was a poor carbon source for the test fungi.

Effect of sorbose on mycelium. The effect of sorbose in restricting the lateral growth of *Neurospora* spp. and *Syncephalastrum racemosum* on agar medium was noted by Tatum *et al.* (1949). The same effect is evident in stationary liquid media. Microscopic examination of mycelium of several fungi has shown that increased branching is the principal cause of restricted extension of the mycelium. In some cases it appeared that a nearly equal weight was produced in the presence of sorbose even though mycelial extension was restricted.

To determine the effect of sorbose on the growth of *Neurospora sitophila* (wild type) on agar and in liquid media the fungus was grown on 25 ml. portions of the liquid media in flasks and on the same amount of the same media solidified with agar in Petri dishes. The three media contained maltose, maltose-sorbose mixture, and sorbose. At the end

of two days the mycelium had reached the edge of the 90 mm. Petri dish containing maltose medium. Harvests of the mycelium and dry weights were obtained after 4 and 6 days. Data for the 6-day period are presented in Table 6.

The mycelium was harvested from the same agar cultures that were measured. The cultures were steamed for 5 minutes to melt the agar and the mycelial mat lifted out, washed with hot water to remove the excess agar. One set of liquid cultures was steamed before harvest, and another set was harvested without steaming.

These data (Table 6) show that the restricted growth due to sorbose in maltose agar media is not evident in the dry weights of mycelium grown in liquid media. The presence of sorbose in liquid maltose media has no effect on dry weight produced, though it does have a decided effect on the extension and weight of mycelium on agar. The rate of sugar utilization is slower on agar. The authors believe that the dry weight of mycelium produced is a more accurate measure of growth than is extension of mycelium over an agar surface.

Table 6. Comparison of Growth of *Neurospora sitophila* (wild type) on Agar and in Liquid Media, With and Without Sorbose

Sugars	Days	On Agar		In Liquid	
		Diam.	Dry Weight culture steamed	Dry Weight culture steamed	Dry Weight culture not steamed
		mm.	mg.	mg.	mg.
Maltose	6	*90+	78	99	110
Maltose and Sorbose	6	38	29	100	105
Sorbose	6	12	4	6	1

*90 mm. after 2 days.

Of all the fungi tested to date, *Endoconidiophora fimbriata* is one of the most sensitive to sorbose. A severe toxic effect was seen when the mycelium was examined microscopically. In maltose medium the hyphae are slender and branching is rather sparse (Figure 5). But in sorbose media the hyphae are thicker and excessively branched (Figure 6). Closer examination showed that the majority of the hyphal tips were killed. Branching usually occurred back of the killed cells. Frequently these branches also were killed and the branching continued (Figures 7 and 8). Staining of the mycelium for a few minutes with phloxine facilitated counting the living and dead hyphal tips. The stain is absorbed more quickly by the dead cells. Results of these counts on three fungi severely inhibited by sorbose are given in Table 7.

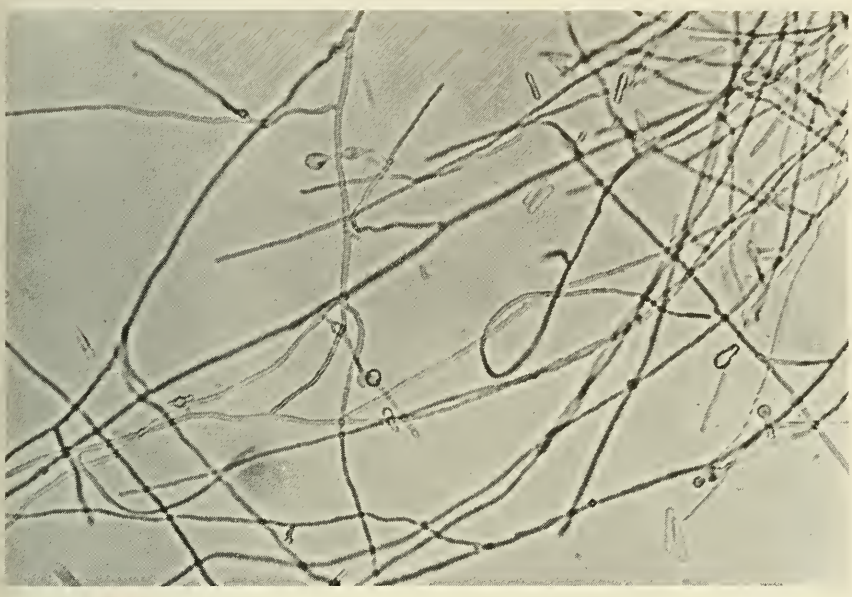


FIGURE 5. MYCELIUM of Endoconidiophora fimbriata grown in liquid media. The extensive mycelium produced in maltose medium (approximately X 100).

FIGURE 6. THE COMPACT MYCELIUM showing excessive branching and many dead hyphal tips produced in sorbose medium. (approximately X 100).

FIGURES 7 and 8. KILLED HYPHAL TIPS of Endoconidiophora fimbriata growing in a sorbose medium (approximately X 450).

Table 7. Number and Percentage of Dead Hyphal Tips of Mycelium Grown in Liquid Maltose and Sorbose Media

	Days	Sugar	Number of dead tips	Number of living tips	Per cent dead tips
Chaetomium	6	maltose	88	275	22
globosum	6	sorbose	146	144	50
Endoconidiophora	6	maltose	29	169	15
fimbriata	6	sorbose	577	169	77
Sordaria	3	maltose	66	551	12
fimicola	3	sorbose	252	189	57

Relative concentration of sugars. The inhibition due to sorbose tends to increase as the relative concentration of sorbose to maltose or glucose is increased. This is more evident with sensitive fungi and during early growth. When the amount of maltose was kept constant at 20 g. per liter and the amount of sorbose was increased (0, 10, 20, 40 g.), the mycelial weights of *Sordaria fimicola* after 5 days were as follows: 215, 87, 20, 11 mg. The weights of *E. fimbriata* after 5 days with glucose constant at 20 g. per liter and the amount of sorbose increased (0, 1, 5, 10, 20 g.) were as follows: 122, 113, 94, 32 and 10 mg.

These results are similar to those reported by Tatum *et al.* (1949). who found that linear growth of *Neurospora crassa* is reduced when the amount of sucrose is held constant and the amount of sorbose increased. An increase in the concentrations of sucrose increased growth at all concentrations of sorbose.

Table 8. Effect of Autoclaved and Sterile-filtered Sorbose on Growth of 4 Fungi. Data Given in mg. Dry Weight

	Days	Maltose autoclaved	Maltose-sorbose autoclaved	Maltose autoclaved, sorbose filtered
Chaetomium globosum	5	84	12	7
	8	159	35	29
Endoconidiophora	5	170	6	8
fimbriata	8	184	14	11
Sordaria fimicola	5	186	31	53
	8	250	117	181
Aspergillus clavatus	5	55	10	16
	8	194	38	82

Effect of sterile-filtration of sorbose on inhibition. The effect of the method of sterilization of sorbose on growth was tested using a maltose-sorbose medium. In one medium the sorbose was autoclaved

with the maltose, and in the second the sorbose was sterile-filtered and added aseptically to autoclaved maltose medium. The results are given in Table 8.

In no case did the growth within eight days in either medium containing sorbose equal or approach the weight obtained in medium containing maltose alone. Growth in medium containing sterile filtered sorbose was approximately the same as in the medium containing autoclaved sorbose. We may conclude that most of the inhibitory action of sorbose is not due to a toxic product formed during autoclaving of media containing this sugar.

Inhibitory effect of sorbose partially overcome by malt extract. In some of the early experiments malt extract was used as the basal medium to which sorbose was added. When the growth of *Chaetomium globosum* was compared with that on maltose-asparagine medium a marked dif-

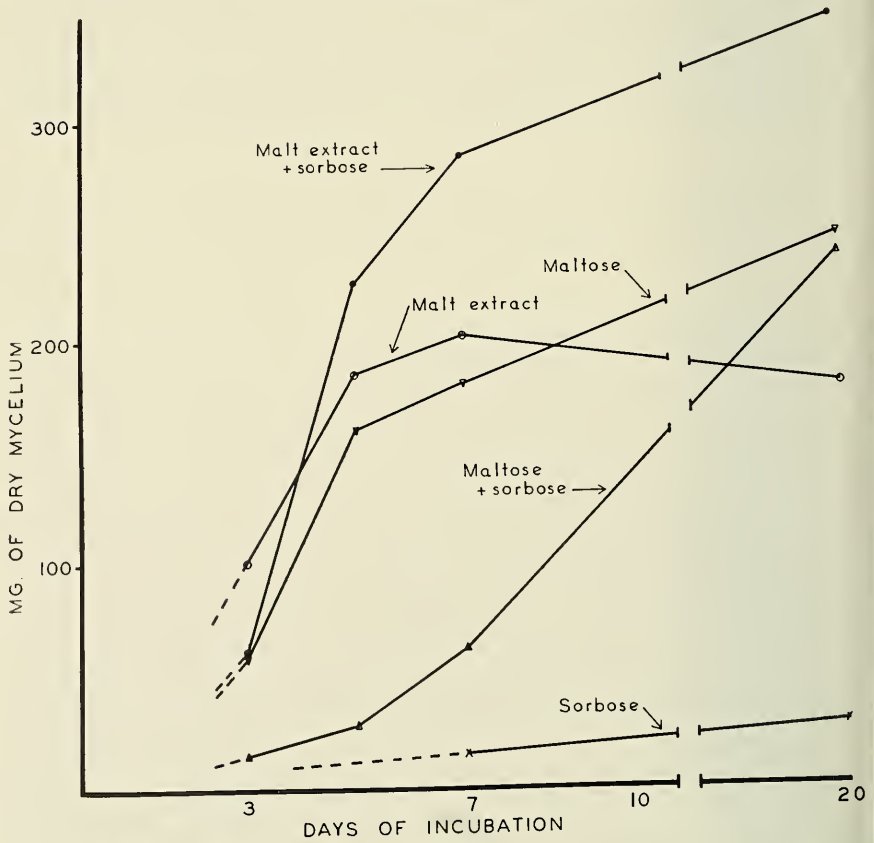


FIGURE 9. GROWTH of Chaetomium globosum in various media showing the effect of malt extract as compared to maltose in overcoming the inhibition by sorbose.

ference was evident (Figure 9). The inhibition by sorbose was somewhat less severe and of distinctly shorter duration in malt extract medium. It appeared that the presence of malt extract partially or completely (depending upon time) overcame the effect of sorbose.

Later a more detailed experiment was carried out in which the amounts of maltose and malt extract were varied, but the total amount of sugar kept constant. Sorbose was constant at 20 g. per liter. Maltose and malt extract alone were used as controls. This experiment was repeated with similar results, the test fungi being harvested at 5 and 10 days. The results for three fungi 10 days old are presented graphically in Figure 10.

Malt extract has a greater effect in overcoming sorbose inhibition of *C. globosum* than when *E. fimbriata* or *S. fimicola* are used. When

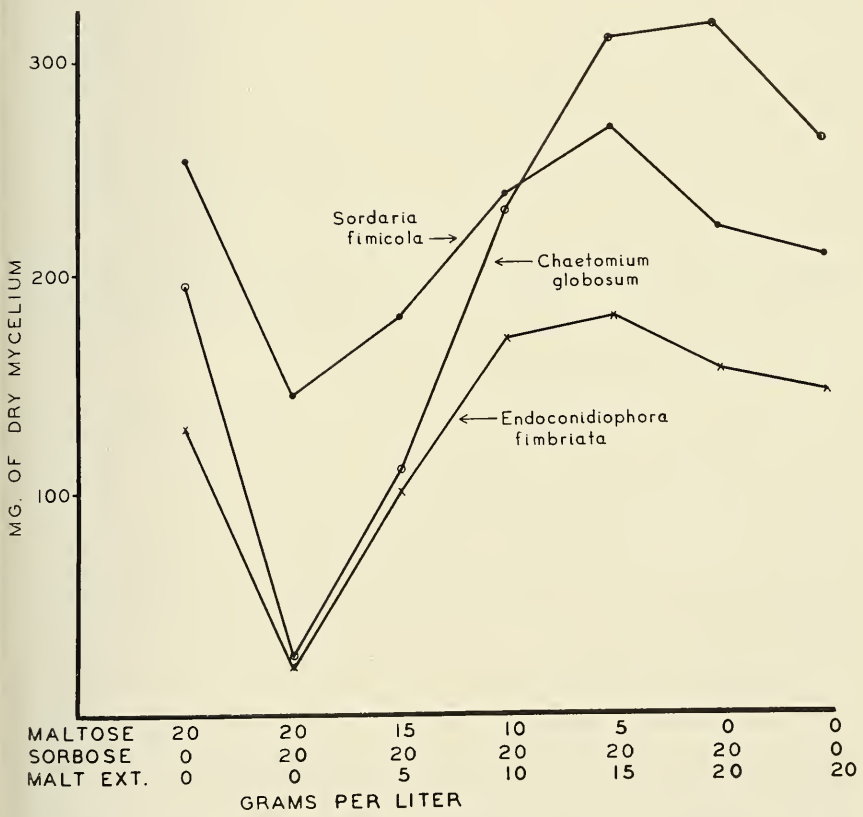


FIGURE 10. GROWTH of three fungi on media varying in relative concentrations of maltose and malt extract after 10 days. Note that increasing the amount of malt extract while decreasing the amount of maltose overcomes the inhibition by sorbose.

sorbose is added to maltose medium there is severe inhibition for longer than 10 days in the absence of malt extract. Growth in malt extract-sorbose medium was less than in malt extract medium at the end of 5 days, but at the end of 10 days this inhibition had been completely overcome and the fungus made even greater growth than in malt extract alone.

It would seem that the stimulating value of malt extract does not lie entirely in its sugar content, but that it would more likely be found in its vitamin or nitrogen content. Yeast extract, when added at the rate of 2 g. per liter to a maltose medium, failed to substitute for malt extract in overcoming sorbose inhibition.

If the virtue of malt extract lies in its vitamin content, it should be possible to remove these vitamins by boiling the malt extract with Norit (activated charcoal). Malt extract, thus treated and untreated, was added to a sorbose medium. Four test fungi (*Chaetomium globosum, Endoconidiophora fimbriata, Sordaria fimicola,* and *Sphaeropsis malorum*) made somewhat greater growth after 11 days on the untreated malt extract than on the treated. However, the Norit treatment failed to completely destroy the activity of malt extract. Similarly, the addition of inositol, pantothenic acid, or a mixture of inositol, pantothenic acid, nicotinic acid, pyridoxine and coprogen failed to affect sorbose inhibition. The addition of peptone gave a slight increase in growth, but this may have been due to its nitrogen content. Tatum *et al.* (1949) also tested the effect of inositol in combination with sorbose and concluded that sorbose did not influence either inositol utilization or inositol synthesis of *Neurospora crassa* or *Syncephalastrum racemosum.*

Effect of nitrogen source on inhibition by sorbose. Since specific combinations of carbon and nitrogen sources are known to effect growth, it was desirable to determine whether growth in media containing sorbose would be influenced by the source of nitrogen present. Media with three sources of carbon were used: maltose, maltose-sorbose, and sorbose. They were used in combination with potassium nitrate, asparagine, glutamic acid, arginine, urea and Casamino Acids (hydrolyzed casein). A portion of the results using *C. globosum* are shown graphically in Figure 11.

Growth on all six nitrogen sources tested was similar when maltose was the only sugar present. The greatest degree of sorbose inhibition occurred in the nitrate and glutamic acid media. Inhibition was intermediate in arginine and urea media, being slightly greater in the presence of arginine. There was little or no inhibition by sorbose when Casamino Acids was the nitrogen source.

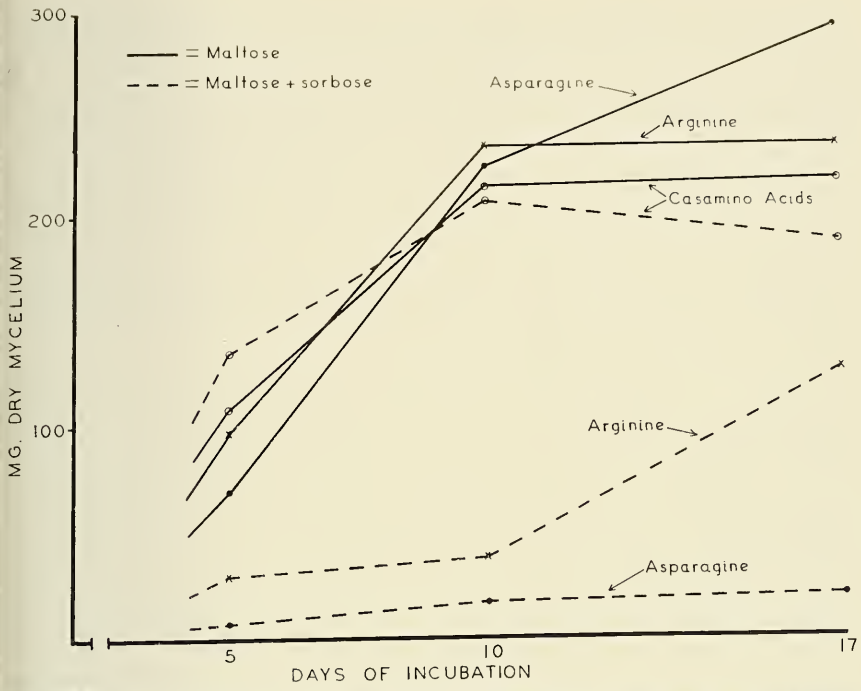


FIGURE 11. GROWTH of Chaetomium globosum in maltose and maltose-sorbose media containing different sources of nitrogen. Note that the inhibition due to sorbose is slight in Casamino Acids medium, intermediate in arginine and greatest when asparagine is used.

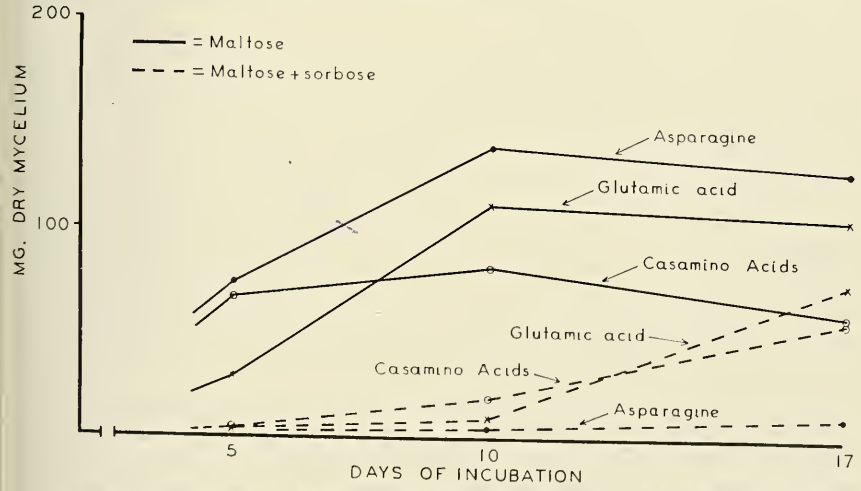


FIGURE 12. GROWTH of Endoconidiophora fimbriata in maltose and maltose-sorbose media containing different sources of nitrogen. Note that the inhibition due to sorbose is partially overcome by the presence of Casamino Acids or glutamic acid.

Variation in the effects of the nitrogen source also was evident when *E. fimbriata* was the test fungus. Some of the results are presented graphically in Figure 12. Sorbose inhibition during early growth was greatest in the presence of arginine and asparagine, and was partially overcome by the presence of Casamino Acids, glutamic acid, and urea.

These results show that the degree of inhibition caused by sorbose in the presence of maltose is influenced by the nitrogen source in the medium. Apparently one or more of the amino acids in Casamino Acids is responsible for the favorable action of this nitrogen source. Asparagine and nitrate nitrogen have little effect in overcoming the sorbose inhibition. The value of arginine, urea, and glutamic acid in this respect seems to vary with the species used.

Effect of acidity of culture medium. The acidity of the culture medium is known to influence many of the activities involved in growth. To determine whether the inhibitory action of sorbose could be overcome by changing the pH, a medium containing yeast extract as the nitrogen source was divided into several lots, each lot being adjusted to a different pH value ranging from 3.0 to 7.0 before autoclaving. Maltose, sorbose, and maltose-sorbose media were used. After 5 days it was evident that acidity had little effect on the inhibitory action of sorbose. The only difference noted was at initial pH 4.0, where no growth occurred in maltose-sorbose medium and 29 mg. dry weight was made in maltose medium. Greater growth occurred both in media at pH values above pH 4.0. Within broad limits we must conclude that the acidity of the medium has little or no effect on the action of sorbose in the maltose-sorbose medium.

Effect of temperature on sorbose inhibition. It has been shown (Barnett and Lilly, 1951) that temperature greatly influences the degree of inhibition by sorbose in the presence of other sugars. The greatest inhibition of *Chaetomium globosum* occurred near 30° C., which is approximately the optimum temperature for growth in glucose, sucrose, and maltose media. In a mixture of any one of these sugars with sorbose the increased inhibition at 30° C. apparently was greater than the tendency for increased growth rate, and as a result there was less growth in the sorbose mixtures at 30° than at 25° C. After 12 days the greatest growth in glucose-sorbose medium was made at 25° C., and in sucrose-sorbose and maltose-sorbose it was at 20° or 15° C.

A more complete summary of these results, including data from additional experiments, is shown in Table 9. In order to present a better picture of the principles shown, averages of the data of duplicate experiments conducted at different times are presented.

These results clearly show that at 30° C. the presence of sorbose inhibits the utilization of glucose, sucrose, and maltose, by *C. globosum*

Table 9. Effect of Temperature on Sorbose Inhibition of *C. globosum*. Average of Two Trials. Dry Weights in mg. T = Trace of Growth, Less than 20 mg.

Temp.	Days	glucose	glucose sorbose	sucrose	sucrose sorbose	maltose	maltose sorbose	sorbose
15° C.	6	35	30	27	35	33	30	T
15° C.	12	73	137	54	108	99	123	T
20° C.	6	94	85	64	54	103	43	T
20° C.	12	233	322	216	186	236	119	T
25° C.	6	188	135	175	17	182	13	T
25° C.	12	329	340	311	67	300	49	T
30° C.	6	235	76	211	10	188	10	T
30° C.	12	350	268	380	48	337	20	T

while at 15° C. there is no inhibition. The degree of inhibition increased with an increase in temperature. In fact, the presence of sorbose increased growth of *C. globosum* at the end of 12 days at 15° C.

In order to show that these effects of temperature are not unique to *C. globosum* and to emphasize the variation in response of different fungi, data on a few other species will be presented briefly. The growth of *Aspergillus clavatus* at the end of 10 days is presented graphically in Figure 13. Two striking points of similarity to the data above are evident. There is little or no inhibition by sorbose in the presence of

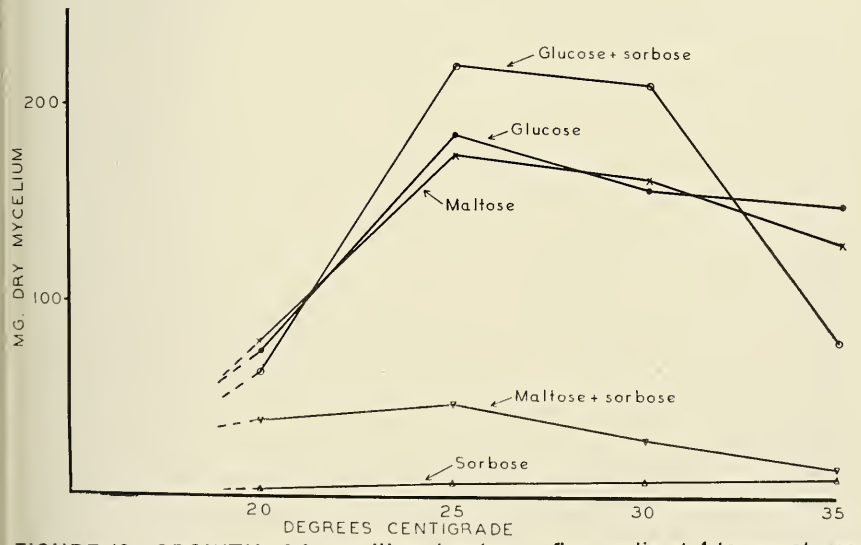


FIGURE 13. GROWTH of Aspergillus clavatus on five media at 4 temperatures, Note that there is little or no inhibition by sorbose in the presence of glucose at 30° C., but in the presence of maltose-sorbose inhibition is great at all temperatures.

glucose, except at 35° C., whereas the inhibition in the presence of maltose is severe at all temperatures.

The inhibitory effects of sorbose on *Sphaeropsis malorum* are not evident except in maltose-sorbose medium at temperatures of 25° C. or above (Table 10).

Alternaria solani (Table 10) illustrates the same principles but shows considerable inhibition in maltose-sorbose medium at temperatures as low as 20° C. Some inhibition is evident in the glucose-sorbose and sucrose-sorbose media.

In contrast with the other fungi tested, *Endoconidiophora fimbriata* does not show the decreased inhibition at lower temperatures up to the 12th day.

It seems that the effects of temperature are common to a number of fungi. The actual temperature that affects the inhibitory action of sorbose may not be so important as the relative temperature in the temperature range for growth. A temperature at or above optimum for growth tends to increase the inhibition. Less inhibition was found at lower temperatures.

TABLE 10. EFFECT OF TEMPERATURE ON INHIBITION OF GROWTH BY SORBOSE OF 3 FUNGI. GROWTH IS EXPRESSED IN MG. DRY WEIGHT

Temp.	Days	glucose	glucose sorbose	sucrose	sucrose sorbose	maltose	maltose sorbose	sorbose
Sphaeropsis malorum								
15° C.	12	104	129	133	101	94	104	17
20° C.	5	56	46	74	70	69	46	T
25° C.	5	153	140	132	101	166	61	T
30° C.	5	122	102	163	124	175	36	T
35° C.	12	22	22	32	22	50	21	T
Alternaria solani								
20° C.	8	36	28	30	24	51	7	5
25° C.	8	107	72	75	56	165	40	15
30° C.	8	214	96	170	119	201	58	14
Endoconidiophora fimbriata								
15° C	12	40	T	35	T	45	T	T
20° C.	5	48	T	61	T	39	T	T
25° C.	5	113	T	119	T	84	T	T
30° C.	5	107	12	126	T	73	T	T

Discussion

The results presented in Part II indicate that sorbose is utilized as a source of carbon by many fungi, although it is apparently inert to others and distinctly inhibitory or toxic to still other species even in the presence of a well-utilized sugar. Even though species of fungi are known to react differently to a given constituent in the medium, it is somewhat surprising to find that a sugar causes such diverse effects. This emphasizes the importance of using a medium containing a single carbon source for physiological studies. One cannot predict the effects of mixed carbon sources on untested fungi. The utilization of one sugar may be influenced by the presence of another sugar.

In most fungi the inhibition of growth caused by sorbose in a mixed sugar medium is not severe. It is evident during the early period of growth, approximately 5 days for most species, and is frequently overcome with prolonged incubation. The killing of the hyphal tips of certain sensitive fungi suggests a definite toxic action. Less sensitive fungi show excessive branching but few dead hyphal tips.

A search for an explanation of the inhibitory action of sorbose has not been completely successful. Sterilization of sorbose by filtration instead of by autoclaving has increased growth only slightly. The degree of inhibition is affected only slightly by the acidity of the medium. The inhibitory action of sorbose is greater in the presence of sucrose and maltose than with glucose. In fact, sorbose may be stimulatory in the presence of glucose and inhibitory in the presence of sucrose or maltose. This suggests that size or complexity of the sugar molecules may be a factor.

Temperature is one of the most important influencing factors. The degree of inhibition is much greater at higher temperatures, usually above optimum for the test fungus. Some fungi make good growth on a mixed sugar medium, such as glucose-sorbose and maltose-sorbose, at temperatures below the optimum, but are strongly inhibited at 30° C. or higher. This suggests that enzymatic activity is the key. It is possible that sorbose affects the synthesis or activity of enzymes required for the utilization of certain other sugars.

From the study of isolated enzyme systems of animal origin, Lardy *et al.* (1950) found that hexokinase activity is inhibited by L-sorbose and D-glyceraldehyde. It was suggested that the inhibitory action is actually due to L-sorbose-1-phosphate which may be formed from both of the above compounds.

Similarly, Spiegelmann and Dunn (1947) demonstrated competition between various enzyme systems responsible for the fermentation of

glucose, maltose and galactose by certain strains of *Saccharomyces cere visiae*.

The influence of the nitrogen source on the rapidity with which sorbose inhibition is overcome is quite interesting. Asparagine, the nitrogen source arbitrarily chosen to be used in most of these experiments, and nitrate nitrogen proved to favor the inhibition. Hydrolyzed casein (Casamino Acids) seemed to counteract the sorbose inhibition more than any other nitrogen source used, although there is little evidence that it favored the utilization of sorbose. Arginine and urea also favored growth of *E. fimbriata* and *C. globosum* in a maltose-sorbose medium.

These results suggest that sorbose may affect the synthesis of an essential amino acid or other metabolite and that the inhibition is not expressed in the presence of favorable nitrogen sources. The nitrogen source has been shown to affect the production of saccharase (DeLey and Vandamme, 1951) and the production of pectinase (Vasudeva, 1930). It is believed that the action of malt extract in partially overcoming sorbose inhibition is due primarily to its amino acid content.

This discussion does not explain completely just how the presence of sorbose in a medium interferes with the utilization of another sugar, but much information has been gained on the influence of nutritional and environmental conditions on sugar utilization. We do not know whether other poorly utilized sugars may have effects similiar to that of sorbose.

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